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C O N T E N T S

DR. ROBERTS: Welcome back to the Scientific Advisory Panel. Today's meeting will extend our discussions on determination of the appropriate FQPA safety factor on the OP pesticide cumulative risk assessment.

I would like to start out as we did yesterday by introducing our designated federal official, Mr. Paul Lewis, and ask him if he has any announcements or instructions for the panel today.

MR. LEWIS: Thank you, Dr. Roberts. And welcome, everyone, to our second day of this FIFRA Scientific Advisory Panel meeting. I just want to again review for the members of the panel and the public that this meeting follows requirements of the Federal Advisory Committee Act. Such that being the case, all materials are available to the public in our public docket. Some major background materials are available and posted on our scientific advisory panel website.

Thank you, again, to members of the panel and for the public for participating in

today's meeting. I'm looking forward again to very challenging and interesting dialogue that will occur during the course of today's discussion.

Dr. Roberts.

DR. ROBERTS: Thank you, Paul.

I would like also to introduce the panel in part because we may have some members of the audience who weren't here yesterday and also because we have two members of the panel that are joining us today.

So let me again ask as we did yesterday beginning to my right, which I guess will be Dr. Hattis this morning, and ask each member of the panel to state their name, affiliation and area of expertise, and we'll just go around the table in a counterclockwise fashion.

Dr. Hattis.

DR. HATTIS: Dale Hattis, Clark University. I'm a risk analysis modeler. I specialize in issues of variability and uncertainty, and I particularly have done some work on pharmacokinetics comparing children of

various ages and adults based on pharmaceutical data.

DR. POPE: I'm Carey Pope from Oklahoma State University. My area is neurotoxicity, neurotoxicology of organophosphorus compounds.

DR. SULTATOS: My name is Les Sultatos. I'm from the department of pharmacology and physiology at New Jersey Medical School. And I'm a pesticide toxicologist.

DR. ELDEFRAWI: Amira Eldefrawi. I'm a professor in the University of Maryland School of Medicine, department of pharmacology and experimental therapeutics. My expertise is in neurotoxicology, my specialty, and with a focus on insecticides and also toxins.

DR. BIGBEE: Good morning. My name is John Bigbee. I'm from the Virginia Commonwealth University, department of anatomy and neurobiology. My field of interest is developmental and noncholinergic roles for acetylcholinesterase, the noncholinergic mechanisms that regulate morphogenic events during

development.

DR. REED: I'm Nu-May Ruby Reed. I am from California Environmental Protection Agency. I'm a staff toxicologist in the department of pesticide regulation. I do pesticide risk assessment.

DR. HARRY: I'm Jean Harry from the National Institute of Environmental Health Sciences. Expertise is in the area of neurotoxicity.

DR. MCCLAIN: I'm Michael McClain. I'm a toxicologist. I have spent most of my career in the pharmaceutical industry doing pharmaceutical development. I have worked for Hoffman LaRoche for 28 years. For the last three years, I have been working as a consultant in toxicology doing mostly pharmaceutical development, and I have my consulting company, McClain Associates.

DR. LAMBERT: I'm George Lambert from the Environmental Occupational Safety and Health Science Institute at U of BNJ (ph) and Rutgers, and I am director of the childhood

center for neurotoxicology and exposure assessment. And I'm a pediatrician neonatologist, pediatric environmental health specialist.

DR. MATSUMURA: I'm Fumio Matsumura from the University of California, Davis. My area of expertise are molecular toxicology on the pesticide toxicology mode of action. My Ph.D. thesis a long, long time ago was on malathion. That's how I started. And this topic is my interest.

DR. NEEDLEMAN: I'm Herbert Needleman. I'm professor of psychiatry and pediatrics at the University of Pittsburgh. And my area of interest is in neurotoxins in child development.

DR. THRALL: Good morning. I'm Mary Anna Thrall. I'm a professor of veterinary pathology at Colorado State University.

DR. PORTIER: Good morning. I'm Chris Portier. I'm the director of the Environmental Toxicology Program at the National Institute of Environmental Health Sciences.

DR. ROBERTS: My name is Steve Roberts.

I'm a professor at the University of Florida in toxicology and serve as the director for the Center for Environmental Toxicology there.

And it's also my pleasure to serve as the chair for today's panel.

We have with us again this morning, I'm pleased to say, Ms. Sherell Sterling, who is the acting director of the Office of Science Coordination and Policy, as well as Ms. Marcia Mulkey, who is the director of Office of Pesticide Programs.

Good morning. And I wanted to ask you if you had any comments or anything for us before we launch into the questions today.

MS. STERLING: For me, just good morning. Welcome. Thank you again, and we look very much forward to the discussions that we're about to hear.

MS. MULKEY: And I will also limit myself to a greeting and thanks, although I want to offer specific and special greetings and thanks to those members who have joined the panel since I

had the opportunity to say something similar yesterday morning. It is very nice to have you here too.

And also to tell you how very much we at the agency are looking forward to today's discussion among you.

DR. ROBERTS: Thank you.

Dr. Dellarco, will you be posing the questions to the panel today?

DR. DELLARCO: Yes, I will.

DR. ROBERTS: Good morning. Do you want to go ahead and begin with the first question?

DR. DELLARCO: We have asked questions under three topic areas that concerns the common mechanism, again, our analysis's focus, on the inhibition of acetylcholinesterase.

And the first question concerns the role of acetylcholinesterase in development.

Question 1.1 says, please comment on the extent to which the report adequately summarizes the current state of knowledge.

Does the scientific evidence support the

conclusion that perturbation of the cholinergic nervous system during development by inhibiting acetylcholinesterase can potentially lead to deficits in the structure and function of the central and peripheral nervous system.

DR. ROBERTS: Dr. Bigbee, I realize you just got here, but can you lead off our discussion in response to this question?

DR. BIGBEE: My interest in this, in cholinesterase, is its noncholinergic role in neurodevelopment and how it can function as an adhesive protein during development.

And this adhesive function of acetylcholinesterase is entirely independent of its cholinergic ability. Complete elimination of its activity does not perturb this ability to promote axonal (ph) outgrowth, neuronal migration and also to some extent neuroproliferation.

And so I guess my first comment, my first question is this idea of common function, and that since not all OPs are the same, that, in our studies we have shown that different

inhibitors, if you treat developing systems mostly in vitro with inhibitor compounds, that different inhibitor compounds have very, very different effects on this morphogenic ability of acetylcholinesterase.

And the reason for that, we propose, is because these inhibitor compounds perturb an adhesive domain on the surface of acetylcholinesterase and thereby prevent its morphogenic abilities.

So a question that I have or a concern would be that the different OPs and their different structure as they interact with the cholinesterase molecule might all produce inhibition, but, because of their different structure, could potentially change the configuration of the molecule.

And by changing the configuration of the molecule, could potentially alter this surface adhesive domain and thereby affect this morphogenic ability of AChE.

And I think it would be an interesting

and an important discussion to have in the report this potential difference or potential effect of the different OPs because of their structure in affecting this surface domain.

DR. ROBERTS: Dr. Eldefrawi, did you have any comments that you wanted to add on this?

DR. ELDEFRAWI: Well, I was delighted to communicate when I came in this morning because I really didn't know much about adhesion molecules. So I'm anxiously waiting to hear that.

In addition, definitely, children need more protection. And because they are exposed more to organophosphates whether playing in the dust or in their homes or in the gardens or proximal planted trees or flowers, therefore, if the exposure is more, then they are more liable to have brain effects than in the adults.

DR. ROBERTS: Dr. Pope.

DR. POPE: The cumulative risk assessment of organophosphorus anticholinesterase is based on their common mechanism of toxicity. Even though it has been shunted around here about

cholinesteration inhibition, most people realize it is not just cholinesterase inhibition. There is a sequence or a cascade of steps that are important and can be modified.

Anyway, that common mechanism is phosphorylation of the enzyme leading to accumulation of acetylcholine and consequent cholinergic signs of toxicity.

Acetylcholine and acetylcholinesterase have been proposed to play a role in the development of the nervous system. A possible adverse effect of the OP anticholinesterases is therefore abnormal neurodevelopment.

Section 2 A of the report adequately describes the available information regarding the roles of acetylcholine and acetylcholinesterase in neurodevelopment. That's one of the questions.

The scientific evidence does not in my opinion, however, provide a strong support for the conclusion that perturbation of the cholinergic system during development by inhibiting acetylcholinesterase can lead to deficits in the

structure and function of the nervous system.

As stated in the report, neuromodulatory roles for both molecules were proposed decades ago. Of particular importance to the risk assessment of OP toxicants, more recent information suggests that some OP inhibitors can modify neuronal growth in vitro.

It should be stressed, however, as noted in the report that some anticholinesterases do not apparently have any effect on neurite outgrowth.

Some studies suggest that neurodevelopment may be affected in vivo by some OP toxicants. Most of these studies utilize unrealistic exposure conditions such as exposing animals to chlorpyrifos and 100 percent DMSO. And thus, the relevance of such of these effects are uncertain.

These findings general suggest, however, that such neurodevelopmental changes are not tightly coupled to inhibition of acetylcholinesterase activity per se, and thus do not constitute endpoints elicited by the common

mechanism of toxicity.

And I think further consideration of the cumulative risk assessment process is therefore not warranted if the risk assessment is based on the common mechanism.

DR. ROBERTS: Dr. Brimijoin.

DR. BRIMIJOIN: I think Dr. Pope has his finger on a key issue here.

I mean, as the question is worded, the answer has to be yes. The question is strictly worded here as, does the scientific evidence support the conclusion that perturbation of the cholinergic system during development by inhibiting AChE can potentially lead to deficits in the structure.

It's really asking is there enough evidence out there for us to consider that this is a large enough unknown.

So just as it is flatly stated, I would have to say the answer is yes. But Dr. Pope is absolutely right, I believe, in indicating that the evidence falls way short of what is needed to

demonstrate that the simple inhibition of AChE and a resulting buildup in acetylcholine itself is or likely to be pure and simply a factor that would perturb neurodevelopment.

In that way, what he said is absolutely right. And he has properly brought the discussion away from the fascinating but still speculative basic science down to the question of what are the implications of this science for this cumulative risk assessment in terms of a common mechanism of action.

I guess I would qualify this, not that we need further complexity, but I would qualify what Dr. Pope just said by saying that it is -- we're moving just slightly away from the explicit focus of the defined common mechanism when we talk about agents that might exert toxicity through their actions on the very same molecule within a few anstroms, in fact, of the active site.

We're not talking about actions of these compounds on totally unknown or hypothetical entities in the nervous system, but the same

protein.

And frankly, if we had enough data to show that even a subset of the OPs, because of the way they interacted with AChE, were indeed putting the organism at risk for developmental abnormality, in that case, I would have to say that although it isn't maybe within the actual letter of the statute or charter that we have here to focus on AChE inhibition, in that case, I think we would immediately want to broaden the definition of common mechanism to include this type of action.

So it is because of that that I would be hesitant to say, well, the evidence is too weak to even consider this as a factor.

And as Dr. Bigbee, I apologize for missing his presentation, but I know fairly well the science that he is presenting and am convinced of its relevance, as Dr. Bigbee is pointing out, some molecules are going to interact with AChE in such a way that they may in deed affect its associated functions, which I'm at least an

agnostic on this. I think that there is a very strong possibility it has associated functions.

For all that, I think that the jury is out, but I would urge us to keep the idea that perturbation of AChE broadly speaking by at least a subset of the OPs has potential for being a developmental risk.

DR. ROBERTS: Thank you. Let me ask the last associate discussant for opinion, and then we'll open this to panel discussion.

Dr. Harry, what is your opinion on this.

DR. HARRY: I'm not sure I can follow those guys.

I'm thinking a little more in the document. There has been a lot of guidance here on things to put in.

And while you can always make a suggestion that there can be more in the document and maybe some of these other issues should be raised within this first part of the document itself, I think it clearly lays out that there can be potential, but it doesn't necessarily

demonstrate it.

And you might want to take the opportunity to take a couple of the issues that were raised, put a few more references in that are the basic biology behind why we would assume this would be happening.

As far as it goes into the examples of the chemical specific that you are going after right now, I think does a very nice job of presenting those.

So my comments go from concepts to more details on the report. But I think with just a little bit of tweaking of a little additional references and background it covers most of those issues.

DR. ROBERTS: Let me open this issue, then, to discussion among the panel at large.

Dr. Lambert.

DR. LAMBERT: From what I hear and I know, it appears that OPs can act through this pathway, through this mechanism and cause toxic effects.

The question that I don't think is asked is is this the only way that it can occur and is this going to be a biomarker sensitive enough and specific enough to identify the risk of OP for children.

And I think that's surely not proven here.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: I'm not sure how to state my question, because I have a question to the panel about the question. That's where I'm a little lost.

The question asks, is it reasonable to assume, and you are saying there is potential evidence. I think from the point of view of EPA, and I'll speak for myself, but I think I would like to have some discussion about the weight of the evidence in support of that assumption.

Is it zero evidence, is it some evidence, is it fairly strong and emerging evidence?

The reason for that, I think, again,

from my perspective, is thinking about what FQPA requires. It is a question of stating that this is not possible or the strength of the evidence that this is not possible is fairly strong, that would lead to the use of not a 10X or not a 3X for that particular aspect.

And so while we had some interesting debate on various parts and pieces of it, I would like some discussion of what the overall strength of the evidence, what you think it would be.

DR. ROBERTS: Dr. Brimijoin.

DR. BRIMIJOIN: Well, I don't know if this will satisfy you, Dr. Portier. Let's just maybe very briefly recapitulate some of the pros and cons. What I see is there is a set of data largely from in vitro work that do suggest a key potential to disturb -- of some of these compounds at least, a set subset of them, to disturb neuronal development with implications that it might extend to the brain, the actual and the intact animal or child. And then there is some opposing evidence.

Maybe Dr. Bigbee will want to add to this. But I would list some of the evidence in favor of this idea as, and I apologize for reiterating what he may have said, findings by Lawyers Group (ph) and Bigbee and others that in vitro systems, a subset of these compounds really do in a fairly profound way affect neurite outgrowth.

Secondly, it is antedating that work or studies by neurobiologists such as Mume and Poo (ph) that show that acetylcholine has important effects on axonal guidance as neurons are developing and growing. So you could expect that marked perturbations in acetylcholine levels locally would be potentially disturbing.

Thirdly, there are the associated, the radical changes in cholinesterase expression at key developmental windows. I mean, they are associated with key developmental events in the brain.

Fourth, there are observations by several groups, including mine, a variety of means

of suppressing the expression of acetylcholinesterase that cause fairly substantial changes in again the growth properties of individual neurons or neuronlike cells in tissue culture.

There are the observation by Slotkin and his group which I don't think are overwhelmingly solid, but, on the other hand, they cannot be dismissed, that there are small but very persistent and profoundly disturbing changes in DNA protein expression patterns in the brain after doses that can be characterized as maybe not environmentally relevant but on the other hand aren't associated with a whole lot of measurable direct effect in the brain so it didn't seem to get too much percentage inhibition to get these effects.

There are also developmental changes mentioned in the document here in fruit flies resulting from genetic disturbances or knockout of genes. All of that is on one side.

Against it, though, is the remarkable

persistence of at least apparently normal development or hardly -- nothing like the radical change that you might have anticipated, I anticipated, from the knockout in mammalian system.

So that ability of a mouse that is totally lacking in AChE to develop an actual brain, and I looked at these brains -- I suppose if I wanted -- I don't know why I wasn't smart enough to decide it was worth publishing our observations that we couldn't find any abnormalities, I tried very hard to find structural neurochemical abnormalities in the brains of the total knockouts. And there's nothing obvious.

So that certainly tells me that in the mammalian nervous system, probably in children, there is a huge potential for at least compensation for what may be an auxiliary developmental function that is disturbed when the enzyme is out.

So it is a mixed bag. And if you forced

me to make a decision, and I think we are in a position or EPA is in a position of having to make decisions, I think there is enough concern that at least some OPs will have in common an ability to affect development by their actions.

DR. ROBERTS: Dr. Portier would like to respond and then Dr. Bigbee.

DR. PORTIER: One quick question for you. I did go back last night and look at the knockout animal papers, in Chi's (ph) paper.

In Chi's paper, you are right. They note absolutely no abnormal pathology in the brain anywhere.

But they do note that the nol (ph) azygous animals begin to radically shake at three days of age and start to actually walk in circles and have abnormal gate very rapidly so that the lack of seeing the pathology from OPs in animals does not in fact preclude the lack of a development -- behavioral or developmental effect.

Is that correct?

DR. BRIMIJOIN: It is certainly true,

and, of course, this is all about, I think, Dr. Harry and Dr. Padilla and others, Carey Pope, would probably stress, the fact that our ability to detect the consequences of minor disturbances in brain structure and function is still limited.

The early neurotox studies were based on does the animal still have a head, can it walk at all, that kind of thing. And we're a long way from getting to what would the animal's SAT score be.

And Dr. Slotkin's group has shown us that we have to look a little farther than just see what's the size of the hippocampus if we want to pick out changes.

So I think as neurobehavioral studies become more sophisticated, there is a potential discover, things that aren't immediately obvious to the untrained eye but are, nonetheless, of profound importance. That's less than a dooms day scenario.

What do I really believe? I really believe that acetylcholinesterase has a minor role

in formation of brain structure. That's just a gut feeling.

DR. ROBERTS: Dr. Bigbee?

DR. BIGBEE: I think something that Dr. Brimijoin said as far as a potential minor role, the -- and it gets to the point that the literature supports the noncholinergic role for acetylcholinesterase in a couple of very well-defined systems, not necessarily throughout the entire neuraxis.

So that the Dorthru Ganglion (ph) system and the Thalamocortical Projections are uniquely high in this development spike of acetylcholinesterase.

And those two systems have been probably the most mind experimental protocols.

And so it is not like it is all throughout the entire nervous system. These two systems are uniquely showing this high developmental expression.

So a total brain acetylcholinesterase activity may not completely give us a picture of

what is happening in some specific subsections or some specific systems.

I think it's important to continue to point out that this developmental role or this structural morphogenic role is completely dissociated from the enzymatic activity of the protein that studies that have point mutations where they have eliminated the activity or in some certain -- some inhibitors, that measuring the enzyme activity may not be the best measure of measuring this morphogenic role. And I think that's an important point.

Another thing about the knockout systems that always worries me a little bit is that the animals that do survive are those that have been clever enough to figure out a way to get around a knockout.

It is a little bit dangerous sometimes to assume that the animal is somehow -- that the acetylcholinesterase, to put a function on it just because it has been knocked out developmentally, experiments where once the animal has committed to

its expression and then knock it down by antisense technology or conditional knockouts are perhaps a little bit more telling about that.

But as Steve was saying, too, I think that the role, this developmental role is probably a very subtle difference in that it has potential for the axonal growth guidance and steering. But it is certainly not some of these more growth morphological, like Steve said, without a head sort of structures.

But I think it is important to keep in mind that we really are talking about two independent parts of this molecule. It's a multifunctional, multidomain molecule. One is its catalytic activity and one is this adhesive morphogenic role.

DR. ROBERTS: Dr. Bigbee, not to put words in your mouth, but in your opinion, you think that the potential neurodevelopmental effects of OPs, or ones that have been observed, are more likely to be due to the noncatalytic -- interactions with noncatalytic portions of the

molecule?

DR. BIGBEE: I think that is one. Then the other would be by having an excess of acetylcholine developmentally can also have its effect through acetylcholine receptors.

So if we're talking just about the acetylcholinesterase molecule itself, the effect there is on this adhesive domain, I believe.

DR. ROBERTS: I believe Dr. Hattis was next and then Dr. Eldefrawi.

DR. HATTIS: I think -- when I read it, I'm not an extensive expert in this area, but the discussion I think is not unreasonable as it stands as a marshalling of the qualitative evidence for concern about cholinesterase inhibition in developing babies and young children.

And if anything, my concern is enhanced by the presence of these other mechanisms of effect, the effect by way of increasing the acetylcholine levels transiently or on a longer term basis with possible consequences for receptor

adaptation and the adhesion properties, where it may in fact not be directly a function of the inhibition of the catalytic activity itself but at least this is a set of molecules that is known to bind irreversibly to that enzyme, and so it is of greater suspicion than your random set of other chemicals that happen to be floating around in the environment.

So at least my index of suspicion is raised about the chemicals even if it turns out that important aspects of their activity is not captured by the raw inhibition potency. It still gives me enough uncertainty that I think concern is warranted.

I think the discussion needs to be improved, and perhaps this will help enhance the analysis with two supplemental discussions.

First, I think there should be a clear articulation of reasonable hypotheses about which dosimetrics for cholinesterase inhibition could be important for the developmental pharmacodynamic actions.

So I think that one really does need to seriously do an analysis of the pharmacodynamics from the available data and any additional data that can be marshalled.

For example, it is not impossible that the best dosimetric for predicting effect could be some peak levels of cholinesterase inhibition on one day or several days of successive exposure. Alternatively, an AUC measure of the integral of percent inhibition by time could prove to be the closest causally relevant predictor of developmental effects. There are also a few more complicated hypotheses that I'll mention a bit later.

In any event, given each of these and/or other plausible measures of internal delivered dose, I think EPA should discuss the roles of activating versus detoxifying enzymes' activities and other factors.

For example, for measures of acute peak cholinesterase inhibition, I expect that activating enzymes would prove to be very

important for those OPs that need activation.

But the detoxifying enzymes such as the esterases will be less important. The opposite would tend to be the case if AUC integrated percent inhibition by time over an extended period of dosing is more important for causing developmental effects.

In that case, activating activity would be somewhat less important and detoxifying enzyme activities for both parent chemical and the active intermediates would tend to be more important.

The in vitro data I think -- that you just mentioned I think can contribute to this discussion if analyzed quantitatively.

What dose by time metrics for the cholinesterase inhibition best explain the effects that can be observed that are thought to be related to developmental changes in vitro.

It might be a lot quicker to get information on that subject. And it's a subject I think that has not been as fully explored in the document as it perhaps could have been if in fact

the in vitro data contained a bunch more quantitative measures of both cholinesterase inhibition and duration that could be inferred.

DR. ROBERTS: Dr. Eldefrawi and then Dr. Needleman and Dr. Thrall.

DR. ELDEFRAWI: I'm going to talk about my special expertise, which I did before yesterday. And that is neurotransmitter receptors.

That included the first receptor ever to be purified about 30 years ago. We purified the nicotinic acetylcholine receptor. These are large size receptor, 25,000.

And when it is activated, it opens its central channel. And then if the dose is very high, the acetylcholine dose, it changes confirmation right away and closes the ionic channel.

On the other hand, the muscarinic receptors are much smaller, (inaudible) 100,000.

And they don't desynthesize that fast. What they do is downregulate their numbers so that

they can fight the excess effect of the acetylcholine that is released by the nerve.

DR. ROBERTS: Dr. Needleman.

DR. NEEDLEMAN: The question divides into two parts. Doesn't it?

The second part is, given the scientific evidence, is it reasonable to assume that perturbation of the cholinergic nervous system leads to deficits in the structure and function of the central and peripheral nervous system.

The answer is, unequivocally, yes, it does.

The first question is, please comment on the extent to which the report adequately summarizes the current state of knowledge.

What we just heard this morning is that it does not adequately summarize the current state knowledge.

This problem belongs in the realm of behavioral teratology. It is a field that has been around for 60, 70 years.

And the principles of that are at lowest

dose, the most sensitive measures of toxicity are in behavior.

And while the document pays lip service to behavioral analyses, it doesn't include it at all in the risk analysis. It just mentions the papers that we have discussed and then goes on to look at a peripheral enzyme to measure a central effect.

Now, it is clear that AChE is a marker for toxicity. In any marker, you are required to furnish certain measures of its utility. That is, its sensitivity, its specificity, its predictive power positive and negative, its correlation with the outcome that you want to know.

None of this has been done. And that leads me to say that there is -- the reason that we're here is to decide if there is enough uncertainty or enough certainty to avoid the obligatory tenfold safety factor.

I think it is clear hat there is enough uncertainty that you cannot do that.

DR. ELDEFRAWI: If I may, I saw the

picture that I brought in yesterday.

For today's invited speakers and guests, I would like very quickly just to explain what that picture is.

You see on way up left corner, there is a cell end that is releasing acetylcholine. However, that's the end of the neuron -- I'm sorry, there is a nicotinic receptor sitting up there around green circles. And the nicotinic receptor when activated, it inhibits the release of the transmitter of that neuron.

Then the big large neuronal end, that does not receive the transmitter. The transmitter in this case is glutamate or gaba.

These studies were detected by electrophysiological methods by my colleague in the University of Maryland, Dr. Edson Albuquerque.

He's an electrophysiologist. So he can measure single events. So the presynaptic preceptors are important, as well as, of course, in most cases, the postsynaptic receptors.

DR. ROBERTS: Dr. Thrall then Dr.

Matsumura.

DR. THRALL: I was just going to suggest that maybe we could make this discussion more simple if we could ask the agency to take out the phrase, by inhibiting acetylcholinesterase inhibition or by inhibiting acetylcholinesterase.

Obviously, that's the biomarker, but it looks like there is a whole and other component to this. If we could just take out that phrase, that might simplify this.

DR. ROBERTS: Yes, but I think sort of -- having that phrase in there has sort of stimulated, I think, some very interesting discussion about the potential for what inhibiting cholinesterase really means.

There is at least apparently two potential modes of action that could be defined as inhibiting cholinesterase.

And there is some implications, I suppose, in the risk assessment in terms of which of -- the weight of evidence, which of those is more plausible because, of course, the potency

estimates and everything are based on the catalytic activity of the enzyme, which is one mode of action.

DR. ROBERTS: Dr. Matsumura I think is next and then Dr. Pope.

DR. MATSUMURA: I basically agree with Dr. Needleman's statement, that we would like to look at more behavioral results and analysis in the final document.

Certainly, there must be some data where -- a generation treatment on all those -- at least some doses to show that some test has been run to look at some sophisticated changes.

I agree with Dr. Pope's position as well, that the roles of behavioral changes may be so subtle and that we are a little worried about.

I have been working on the autism in the last two years. They are really, really dedicated. You can't find anything really a little bit effect on noxtocian (ph). I'm not even sure whether that can really be tied to gross behavioral problem, which we just can't find the

molecular biological clue about the autism. So I'm on the side of a little more cautious.

But at the same time, I would like to look at the perspectives once more. And if we look at the chlorpyrifos, looking at those two papers by Slotkin's group, .1 milligram per kilogram I see effects in behavioral as well as the other effect.

So if you do that, then when you look at the probablistic model, at the 99.9 percent, it is one hundredfold margin, so the difference safety factor if we accept that is the most sensitive method.

So the question is, is this one hundredfold enough to cover that unknowns. And I would like to really look at the overall perspectives. And certainly the agency did a pretty good job really looking at the old types of the exposure.

So the point to me is that if there is one hundredfold difference in the 99.9 percentile, the question really is this real. There are lots

of other types of options in the intricate on one side. The other side is that, yes, we agree with the regulatory agencies that they have to make some decision and that we have to ask really to check is this real.

Are we really close enough, 100 times safety factor here. Is that in the reason that we should be really jumping on or not.

So that's a question I would like to raise.

DR. ROBERTS: Dr. Pope.

DR. POPE: There has been a lot of excellent points brought up on in the discussion on this topic. Some of these points are well taken.

Dr. Thrall's suggestion that we might be able to alleviate the problem by getting rid of the phrase or the idea of inhibition of acetylcholinesterase I think is the pivotal part for me. The way I see it, that's what the whole process is based on.

And while the role of acetylcholine

itself as a neuromodulator, I can see that as being part of the process.

However, the point I was trying to make is that if you have compounds that are inhibitors of acetylcholinesterase that do affect some of these processes in vitro and others that are very potent cholinesterase inhibitors that don't, then I don't see how this could be part of the process of cumulative risk assessment based on cholinergic toxicity.

DR. ROBERTS: And by inhibiting cholinesterase, you mean the asteratic part of the molecule --

DR. POPE: That's what I mean.

DR. ROBERTS: We have to be very careful about our semantics and what we're talking about because the cholinesterase as a protein versus -- most of our methods in our potency assessment is based on the asteratic attributes and activities in the molecule as opposed to perhaps some other functions in the molecule.

Dr. Brimijoin.

DR. BRIMIJOIN: Just a very small addition. Basically, I agree with you, Carey, although, I still wonder if the evidence gets more solid whether we'll have to broaden the notion of what is a common mechanism. But right now I think we have to go with what we know happens.

But I would like to make the small point, I think Dr. Bigbee will agree with me, say so if you don't, John, that this sort of other action on the acetylcholinesterase molecule, which I think we're imagining might involve a disturbance of interactions, that protein and other protein molecules in the vicinity maybe is not impossible, but it is very unlikely that any of these pesticides could have that kind of action without also causing AChE inhibition.

So that putative site is so close to the catalytic gorge that to date any molecule that is known to interact with that area of the surface, including the snake toxins that can't even get into the active site, do have a profound inhibition of acetylcholinesterase activity. So

we would expect that to be a common feature.

It is possible that somebody may discover a weird molecule in the future that can block these adhesive functions by just sort of coming near that zone or just disturbing the interaction without preventing access of the substrate, without disturbing the function.

But that's very unlikely to happen with an OP.

DR. BIGBEE: I agree. There is really no evidence that an OP is binding to that or interfering with the site.

DR. ROBERTS: For the record, that was Dr. Bigbee.

I have Dr. Harry next and then Dr. Sultatos and then Dr. Hattis.

Let me remind the panel. I think what we're really sort of being asked here, at least in the second part of this, is this an endpoint that is plausibly related to the mode of action that is being addressed in this cumulative risk assessment.

I think they need a pretty clear articulation from us in terms of does the science support linking this endpoint with this mode of action.

Dr. Harry.

DR. HARRY: That was somewhat of my point that I was going to make in the sense that I think the discussions that have gone on leads into the first question about is there an adequate representation of the scientific knowledge and data for that.

And the agency could sit there and write five or six review papers if we start going into all of these things. I do think they address these compounds rather nicely. A little more of the background could help, as I said, in the original comment.

But the other question that is here is somewhere along the line I assume the advisory panel accepted this as a biomarker of a common mechanism of action in the adult, right, for looking at these pesticides.

Now, the question is, we're now asked to make the assumption that that will cross over to the developing organism. There seems to be some discussion there.

But to come back and say is this a viable mechanism by which they can look at to do a cumulative risk assessment given the fact that they have also looked at each individual one of these compounds for their most sensitive endpoint which has included behavior and everything else.

I wasn't here for the presentation yesterday, and I sort of quickly tried to glance through the slides. But with the questions that have been raised, it seems like we still come back to asking for the behavior.

So I was wondering if I could ask the agency for a question of, if you are looking at these levels of inhibition, what is the relative changes that you see in behavior?

Can you give us some sort of feel for what you see is what you expect to see -- of the data that you have, would you see it higher than

this.

If we can have a framework, that might help address some of the questions that some panel members have.

That may be another question further down, but it seems a framework that is getting in the way of things right now.

DR. ROBERTS: Dr. Dellarco, do you want to respond?

DR. DELLARCO: I'll take the first stab, then I'll ask Dr. Padilla and Dr. Baetcke to add to this.

But in general, what we see in the data that we have when you look at clinical signs, they typically occur at much higher doses than where you can see cholinesterase inhibition. Typically, you can see cholinesterase inhibition occurring at lower doses.

Now, there are exceptions, or you see them occurring about at the same levels. But we don't see the behavioral effects occurring at doses lower than where we can detect significant

cholinesterase inhibition.

I would like to try to summarize what I have heard so far to make sure I understand it. And i want to put it in very simple terms. Maybe it is best we wait until all the deliberations are over with. I'm trying to understand what the panel is saying on this question.

DR. ROBERTS: We're sort of circling around. I'm hoping our opinion is going to become more crystallized as our discussion continues.

So let's let the panel sort of go through that process. And if we're not where we need to be at the end of that discussion, then I would ask you to do that, because I think it is very important that we make our opinion as clear as we can.

Dr. Sultatos and then Dr. Hattis.

DR. SULTATOS: I have a question for, I guess, Dr. Bigbee or Dr. Brimijoin.

Is the adhesive site that we're talking about here the peripheral binding site on acetylcholinesterase?

DR. BRIMIJOIN: Near it.

DR. BIGBEE: And including it.

DR. BRIMIJOIN: Overlapping it on the surface, outer surface.

DR. SULTATOS: Because occupying the peripheral binding site does in fact inhibit acetylcholinesterase. It is just a different mechanism of inhibition. It is an allosteric modification of the active site. So it's not a phosphorylation, but you still inhibit acetylcholinesterase.

DR. BRIMIJOIN: But the reason it is difficult to fold that into the common mechanism is that nobody is proposing that it is the inhibition of the activity that is responsible for the cellular effects.

DR. ROBERTS: Dr. Hattis, then Dr. Portier.

DR. HATTIS: I just have two brief, further comments that I didn't say before.

This first goes to the knockout mouse. In my view, the knockout mouse evidence is

surprising, but doesn't, I think, completely argue against important effects of transient fluctuations of the acetylcholinesterase activity or inhibition, because the transient fluctuations present a substantially different potential for adaptation than in the case of the heterozygous and homozygous knockout mice, which have the opportunity to develop their connections and feedback control processes in a more consistent basis.

Finally, I want to suggest that the mouse with an apparently recovered whole brain cholinesterase activity is not necessarily the same as an unexposed mouse, and could have in fact persisting effects due to the fact that some of its cholinesterase molecules could continue to be inhibited.

Imagine that you have a bunch of synapses where the cholinesterase that were present prior to the exposure and those molecules continue to be inhibited unless they are resynthesized by the same cell.

But new synapses may well have lots of newly synthesized and therefore completely uninhibited acetylcholinesterase enzymes.

And therefore, you are talking about a situation that even though -- if -- you have 10 percent residual inhibition in that situation is not the same thing as if you have just inhibited 10 percent uniformly.

And so, that's part of my concern to develop better dosemetrics. Perhaps one of the neuroscientists either from EPA or on the panel could flush out my understanding of that because I'm not absolutely sure.

But my impression is that the cholinesterase molecules would have to be made within the particular cells that are participants in a particular synapse in order to be working.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: Dr. Dellarco, I need some clarification again. There was a question you got yesterday that sort of we didn't get an answer. We did partially about DNT studies.

As I understand it, you have two DNT studies in hand. Is that correct? Full DNT --

DR. DELLARCO: Full DNT studies.

The report on page 7 in a footnote summarizes the status of the DNT studies. We have already gotten the chlorpyrifos DNT study. That was reviewed quite a while ago and discussed.

We have completed the review of dimethoate. I believe that we have given you that.

Malathion, we have completed the cholinesterase review, but the scientists in our organization are still going over the other measures and the DNT. So that's not available right now.

For methyl parathion, I believe, that's the same situation.

So we have gotten several DNT studies for the cholinesterase data, but not necessarily all the other neurological measures.

And again, the status is on page 7.

DR. PORTIER: I just found it. I didn't read the footnote.

So then in terms of -- again, a clarification issue. In terms of behavioral effects from fetal exposure into juvenile and adult life, the total body of data consists of the DNT studies you have in hand, the Slotkin studies on chlorpyrifos, and a few other --

DR. DELLARCO: And the literature.

DR. PORTIER: -- there's things in other -- not necessarily mammalian systems.

Is that pretty much the gist of the information?

DR. DELLARCO: I think so.

I think that's a reasonable summary of it.

DR. PORTIER: I will note one thing again for the record that I'll put in my response here, that Dr. Sass's comments yesterday about the analysis of the malathion data does concern me.

In looking at those tables in the analysis that was done there relative to the analysis done by Slotkin, Slotkin log transformed the data. In the malathion study, they did not.

Slotkin did an analysis of variance to find these effects, which is a much more powerful, statistical tool. In the malathion study, that did not appear to be done.

I think when you look at these DNT studies for behavioral effects, I would strongly suggest that they be reanalyzed with a log transform and a full analysis of variance so they are comparable to Slotkin's study and can be easily compared across the various OPs.

DR. ROBERTS: Thank you, Dr. Portier.

Dr. Dellarco, we're not there yet, but I'm hoping we can get some closure on this question fairly soon.

Let me ask Dr. Bigbee or Dr. Pope, since they have a lot of experience in this area and have been listening attentively to our discussion.

If either one of them want to volunteer to sort of capsulize our response so far, the short answer.

We have given them a lot of suggestions. I think that there is -- I have heard varying

opinions on the degree to which the report adequately summarizes the current state of knowledge. There have been some suggestions about aspects that need to be added, and we can certainly include that in our report.

But the second question is a pivotal one. Is a very important one. And I think we need to be very clear in how we respond to this.

So not to put you on the spot. Dr. Bigbee, do you think you could sort of capture the --

DR. BIGBEE: I think the key word, and Dr. Brimijoin said this, is potentially. That's the word.

And potentially, it is there. It can cause deficits in structure and function, potentially.

And another thing as far as the behavioral studies, the two major systems that have been looked at are sensory systems.

And sometimes the abnormalities in the sensory system are a little bit harder to

determine than motor systems.

So I just see that great big potentially word there, and I think we -- my main is concern is that there needs to be information, more information in the document as far as our discussion today, but that certainly with the potential there, I think we have to give that a lot of weight.

DR. ROBERTS: So potentially, yes, but potentially not. And the document really doesn't cover the scientific strengths and weaknesses of that -- the evidence for that linkage. Is that correct?

DR. BIGBEE: Yes.

DR. ROBERTS: Does anyone else have a different viewpoint or want to try and summarize it differently?

Dr. Dellarco.

DR. DELLARCO: Can I try to summarize it in really simple terms, make sure that I'm not misinterpreting anything?

DR. ROBERTS: Absolutely.

DR. DELLARCO: From listening to the discussions, particularly the comments that Dr. Brimijoin, Dr. Pope and Dr. Bigbee have made, this is my understanding, that the basis of the cumulative assessment was done on the ability of these 30 OPs to act on the same site of the acetylcholinesterase molecule. And phosphoryly, it didn't. Thus, inhibited (ph).

However, when we moved to the developing system, there may be other actions on that molecule, and there may be subgroups of OPs and how they affect that molecule based on their structural characteristics -- maybe a chemical kind of OP specific kind of thing.

So although we can say we have a common mechanism for cholinergic toxicity, we can't necessarily say for all 30 of these OPs we have a common mechanism for neurodevelopmental toxicity.

However, it's not unreasonable to assume that the inhibition of acetylcholinesterase may not be a bad biomarker of effects because it is -- again, it is affecting -- if it's acting on that

molecule through another action, it is probably going to be inhibiting it in the way that -- in terms of the catalytic function.

So as we look at common mechanisms of neurodevelopmental effects, there may be subgroups there. Is that what I'm hearing?

And this doesn't mean we shouldn't be concerned about neurodevelopmental effects and continue to look at OPs, particularly on a chemical by chemical basis as data continues to emerge and we continue to understand mechanisms and effects.

DR. ROBERTS: I think that's certainly a path forward. Let's see whether the panel agrees with that description and assessment.

Anyone want to weigh in on that? Dr. McClain.

DR. MCCLAIN: Listening to the EPA presentation yesterday morning, I got a much more clearer understanding of how you are actually focusing this.

And once I had that understanding, my

opinion on some of these questions did change. Because I confused, like I think perhaps some other are confusing, the limitations and the focus on the common mode of action, which is the inhibition of acetylcholinesterase, all of the other effects, the developmental teratology, the toxicity, the carcinogenicity and what ever other studies have been done with these compounds would have been included in the risk assessments and the tolerances for each of the individual's OPs.

So I know I was very confused until I heard your presentation. And I think your point, and you have done it versus succinctly, that you have to make the distinction between what you are evaluating on the common mode of action and any other potential toxicity of these 30 OPs that are handled on an individual basis.

And you can't bring in all of the effects of those 30s into this cumulative risk assessment.

So I think the way you have just expressed it now I have a much better

understanding of that yesterday morning. And I think that's the way, the perspective that we need to take on this.

DR. ROBERTS: Other view points? Dr. Brimijoin.

DR. BRIMIJOIN: I want to say something I hope it simplifies rather than complicates.

Despite the evidence that there may be a structural kind of basis for developmental abnormalities caused by acetylcholinesterase inhibitors, in other words, other sites -- other mechanisms than simply raising acetylcholine levels locally, despite that interesting evidence emerging from all these in vitro studies, I'll just say, personally, if you force me to come right down to the question, would inhibition of acetylcholinesterase and a resulting rise in acetylcholine levels in certain regions of the brain have the potential for causing lasting effects on either the brain structure or the function, I would have to say that I already think there is enough potential for that, that enough

uncertainty about that possibility that EPA would be wise to incorporate that into their thinking about what is an appropriate safety factor for the developing organism. Just on that basis alone.

And we must not lose site of the fact that OPs do inhibit acetylcholinesterase.

And one further point of information is that in the knockout mice, the one thing that has been seen that I'm aware of, and I don't know if it has made its way into the papers published yet, but is very substantial and I guess permanent changes in the level of acetylcholine receptors in the brain.

So the animal has adapted, but the brain is different, and in a way that perhaps you and I wouldn't want our children's brains to be different.

DR. ROBERTS: And not to put words in your mouth, but I assume from your remarks that you think that including this endpoint, meaning neurobehavioral effects in this cumulative risk assessment, which is based on a common mode of

action involving cholinesterase inhibition is appropriate based on existing scientific information --

DR. BRIMIJOIN: Yes, I do.

DR. ROBERTS: Dr. Pope.

DR. POPE: Just one quick question to Steve. That's whether the receptors are permanently altered in the heterozygotes or just the homozygotes.

DR. BRIMIJOIN: I wish I knew the answer. I don't, but I think they probably are, but I don't know.

DR. ROBERTS: Dr. Lambert.

DR. LAMBERT: Just a clarification from the agency.

Are we also trying to address that is this going to be the bottom line for assessing the potential developmental neurotoxicology potential of these class of chemicals?

DR. DELLARCO: In the context of cumulative assessment, but just in general?

DR. LAMBERT: Right.

DR. DELLARCO: I think what we're hearing today will be very helpful, not only to how we look at this issue in the cumulative assessment, but how we continue to look at this issue in individual chemical assessments on the OPs.

Does that respond to your --

DR. LAMBERT: I think most everybody around the table agrees that it is an important pathway of toxicity.

The question that some of us have, I think, is is it the most sensitive and specific and is it so sensitive and so specific that will capture risk to the human child.

That's much more difficult.

DR. DELLARCO: The other point I'll raise is that, as stated yesterday, the bulk of these developmental neurotoxicity studies will be in by 2003. We don't have many of them. And we will continue to look at them as they come in and appropriately revisit chemical assessments. We will be looking at that as that data and knowledge

continues to emerge.

DR. ROBERTS: Dr. Portier.

DR. PORTIER: I'm going to agree with Dr. Brimijoin. I think he did an excellent job of summarizing very clearly my views.

And based upon that, Dr. Dellarco, I would argue that waiting for the -- I don't know if you are going to have to put this risk assessment out before you get those DNT studies in 2003, but I would argue that without those DNT studies we don't have sufficient weight of the evidence to argue that there isn't a consistent behavioral reduction that is also potentially linked to the acetylcholinesterase inhibition.

And I think that's a key issue.

DR. DELLARCO: Can I respond?

DR. ROBERTS: Please.

DR. DELLARCO: There is one important premise in the report. And that is the mechanism is the inhibition of acetylcholinesterase. That's the precursor event. And if we account for age-dependent sensitivity, we should account for

the behavioral effects that are associated with that mechanism.

DR. ROBERTS: Dr. Portier will respond, and then Dr. Pope.

DR. PORTIER: I guess I have a difficulty with that question, with that response, since the correction factors you are using across the OPs to develop the overall exposure index are based upon the adult studies and not upon a potential for specific sensitivity in the infant that is beyond the acetylcholinesterase inhibition that led to the toxicity in the adults that you had observed.

And that's the question here, is that whether the neurobehavioral effects above and beyond what occurs in the adult are something that we need to be worried about on a per acetylcholinesterase inhibition measure.

And that's the thing that hasn't been demonstrated because we haven't seen enough DNT studies and behavioral responses to decide whether that is a common difference, a common effect

across many of these OPs or not. That's my opinion on it.

DR. DELLARCO: I have a question for Dr. Portier.

What you are saying is that, although, we have accounted for in the relative potency factors the potential for the young to respond at lower doses to cholinesterase inhibition, you don't consider that adequate because you feel that behavioral effects can occur at doses lower than that?

DR. PORTIER: No. The issue is I don't know.

We haven't established the question of whether a 10 percent acetylcholinesterase inhibition in an infant leads to an equivalent toxicity of a 10 percent cholinesterase inhibition in an adult.

There are indications that a particular inhibition in an infant may lead to a different outcome in an adult than you have ever seen in an adult.

That's an added risk. And it is that issue, I think, that plays an important role in this debate.

DR. ROBERTS: Did you want to respond, or do you want to move on?

Dr. Pope.

DR. POPE: Just a brief comment about Dr. Lambert's question about sensitivity and also the recent discussion here is that generally speaking the acetylcholinesterase, for example, 10 percent toxicity -- I mean toxicity associated with 10 percent inhibition really isn't there.

There is no toxicity associated with 10 percent inhibition. Generally, the synapse has excess enzyme levels, and most people think that there is some degree of inhibition that can be tolerated before you alter cholinergic neurotransmission.

As I say that, I am thinking I have a little uncertainty regarding the very young central nervous system, so I'm not really as confident there. But that is something that

should be considered.

There is generally safety built into the synapse because of excess enzyme.

DR. ROBERTS: Dr. Hattis.

DR. HATTIS: I think when we're talking about the effects of 10 percent inhibition in adult animals on a long-term continuing basis, I think it does beg the question about whether that that's the right dosimeter for predicting effects, these likely developmental effects and, you know, that could in fact have an effect of transient inhibition that could be greater than that that could result from one or a few doses that you wouldn't capture with that chronic, that longer-term measure.

Or, it could be that even a rather modest inhibition, maybe less than 10 percent, in fact turns out to have some marginal change in the numbers of connections that get made or don't get made because of marginal changes.

The developing organism is a situation where lots of things could be at the edge. It is

not necessarily so that we have functional reserve capacity for all of the important cells and all of the important places doing all the important functions.

So I think it is at least an issue of concern to try to do some pharmacodynamics based on either in vivo or in vitro studies. And that's part of the uncertainty -- the relationship between the pharmacokinetic measure -- the cholinesterase inhibition and the pharmacodynamics is I think still an uncertainty that that remains from the current database, despite the fact that one has never seen obvious changes, these behavioral changes that have only been observed a few times as far as I can tell.

And the database is just not very impressive to be able to conclude firmly that 10 percent inhibition in adults is without important effects in -- you know, for this one dosimeter is without important effects in developing organisms.

DR. ROBERTS: About 10 minutes ago we were on the brink of clarity for our panel

response to this question I think after Dr. Brimijoin spoke. So I'm going go back to Dr. Brimijoin to recapture that moment and see if we can come to some closure on this particular question and move on.

Some of the other comments are good comments that people are making, but I think they may fit in elsewhere in our discussion. I would like to sort of move things forward. So let me go back to Dr. Brimijoin and then I'm going to ask Dr. Dellarco whether we have put together a good response.

DR. BRIMIJOIN: I was starting to feel that we were kind of drifting away from the point here. I think we have heard a lot of important points made, but I'm listening very carefully trying to filter it all.

And I really haven't had any input coming in here that seems to -- I'm not hearing disagreement among the panel. I'm hearing all kinds of caveats and finer points being raised.

But I'm hearing a consensus that the

panel agrees with the idea that there is enough information out there, even there is enough information in the document itself, and there is enough information out there for us to have a level of concern that there are, there is a potential developmental risk from the action of OPs to inhibit acetylcholinesterase activity.

And there may be a variety of mechanisms by which other things can happen as well, but there is a level of concern that this exists.

And so in that sense, we have already reached a consensus on the formal answer to the formal question. I think what may be bothering some of the panel members, such as Dr. Portier, is that what was not asked in this question is, oh, well, in fact, I can't find it, strikingly, anywhere in this array of questions put to us, I can't find -- is it there, just a flat question, does the panel agree with the agency's proposal specifically to go with a threefold FQPA safety factor with compounds that are shown to have a certain degree of extra sensitivity.

We aren't asked that. I think we should have been. If we were, Dr. Portier would be, I think, very much on the point to be saying, we're not sure that a amount of additional inhibition here is the same thing in the neonate as the adult.

I think that's a question that does need to be dealt with.

Personally, I think the EPA has struck a middle ground here in saying, yes, we do have to make an FQPA adjustment. Yes, indeed, we do. But maybe not an extreme one.

But as for the purposes of this question, I suggest we have already reached consensus. And it is time to move on to the remaining questions.

DR. ROBERTS: Okay.

Dr. Bigbee, as the report coordinator, for this particular session, do you have a pretty good sense of what the panels's response might be?

DR. BIGBEE: Yes. And certainly, I appreciate everybody's input.

DR. ROBERTS: So basically, as I hear it, the answers to the questions are: There is some other discussion that needs to be added; and is it reasonable to assume it would lead to deficit in the structure and function, the answer is, yes, but there would be lots of sort of qualifications associated with that that would appear in the discussion.

Dr. Eldefrawi.

DR. ELDEFRAWI: I have a stupid question, but I'm interested to know the response, if there is.

How about the old people, not with Alzheimer's or other diseases, but are they as susceptible or more susceptible than the younger people or not. I really don't know.

DR. ROBERTS: That issue was raised, actually, in our last discussion, at our last SAP meeting. And perhaps we can talk about that at the end of this one. But I would like to sort of keep us focused on the questions. That was an issue, however, that was raised at the last review

or go-over on the cumulative risk assessment.

Dr. Needleman.

DR. NEEDLEMAN: Just to amplify what Dr. Brimijoin said, the unasked question in question 1 is: Is the data adequate enough to certify certainty for the prescribed threefold safety factor.

That should, I think, be in the first question.

DR. ROBERTS: I suspect we'll get the opportunity later on to discuss what the appropriate uncertainty factor might be given the various uncertainties.

But I think this was a pivotal question about whether or not this endpoint needs to be included this cumulative risk assessment.

And my understanding, and please correct me if there is any disagreement from this panel, but it seems that the response is yes, this is an endpoint that should be included in this cumulative risk assessment which is based on cholinesterase inhibition.

Is there any disagreement with that?

Dr. Dellarco, have we finally --

DR. DELLARCO: I just want to come back to one issue that Dr. Portier raised about the benchmark 10 response that is being used in the assessment for our point of departure.

When you make uncertainty and safety factor determinations, you have to look at all aspects of the assessment and weigh the biases in the assessment with respect to the input parameters where there is conservatisms and where there is not and make that decision.

So as you think about that benchmark 10 response, I would ask you to consider that, as I stated in my talk yesterday, that's in light of the 10X interspecies factor and intraspecies factor, 100X.

That's considered as a group factor in this assessment.

DR. ROBERTS: Are we ready to move forward to the next question? I would like to at least get through 2.1 before we take a break this

morning.

DR. DELLARCO: We're going to move to our second topic area, which includes the interpretation of the animal studies with respect to age-dependent sensitivity to acetylcholinesterase inhibition.

This is question 2.1. Please comment on the extent to which the report adequately discussed and summarized the current understanding of age-dependent sensitivity to cholinesterase inhibition, the prevailing views in the scientific community concerning the biological factors involved and the role esterases may play as a major factor accounting for the potential increased sensitivity of the immature rat.

DR. ROBERTS: Dr. Harry, would you mind leading off our discussion on this question?

DR. HARRY: I think you are going to end up with a lot of different comments about the same way we did in the last one, because it is asking for whether there is a sufficient amount of information available that you can provide in the

document.

Within the framework of how the document was formed and I think the level at which it was focused on, it gave enough understanding of the differences but maybe not all the details that could be possible that I'm sure other members of the panel can pull out for you to expand upon some of that discussion.

I did have a couple things. And they may cross down in some other questions. Since I'm not on those, I will sort of say them as they will cover over, but I won't expand upon them.

But when I was going through this, one of the things that I was finding it a little difficult, and again, I'm focusing on what you have written in the document, I found it a little difficult to understand how you were handling the detoxification of the animal with the modes that you had, the cumulative dosing versus acute dosing, and then also the rebound or the apparent rebound loss of inhibition going on.

And that may be a dilution factor or

things like that, but I think that that needs to be in the document in a transition to explain that a little more, because right now it is sort of a what-type question and exactly how you are looking at those two endpoints there.

The other -- this may come down on time, but I think it also comes in here. When you are looking at the role of these compounds and what they will do on the esterases to decrease them is the fact that you have very little data and you have very little data at which you can compare quite often as in the dose that was given, the route of administration, the timing of doing the esterases.

So, like I said, other people have more knowledge of the basic biology behind this. I think what was presented in the document was focused on these OPs, the knowledge that you have about them and presented rather clearly.

The problem is you don't have a whole lot of information to be working with. But it did present some concepts that those are being taken

into consideration with risk assessment.

DR. ROBERTS: Thank you, Dr. Harry.

Dr. Sultatos, did you find the discussion adequate or are there things that you think need to be addressed?

DR. SULTATOS: I think there are things that need to be addressed and added. I think the discussion of the biological factors that might result in age-dependent toxicity of certain OPs and specifically the A esterases and carboxyl esterases could be significantly improved by presenting a more balanced interpretation of the available data.

I think the report summarizes evidence that supports important roles for A esterase and carboxylesterase in the increased sensitivity of the immature rat, but it ignores observations or interpretations that might confound that view.

As a result, I think the document overstates the degree to which the mechanism of age-dependent toxicities of OPs are understood.

And I think it is most apparent, at

least for me, with regard to three issues.

First, the document summarizes several studies that have reported correlations between the temporal patterns of development of A esterase and carboxylesterase activities and OP sensitivity.

But it doesn't talk about some of those same studies which have reported a decreased capacity for activation in the immature rat.

It was touched upon a little bit in the presentation yesterday, but there is nothing said about it in the document.

Immature rats do have reduced capacity to detoxify certain oxons, but they also have less oxon present because they are producing less oxon.

So I think this is a confounding factor that needs to be discussed. And it may implicate other factors involved in the differential toxicity between immature rats and adult rats.

It also may have some bearing on one of the later questions when we're talking about or we're discussing possible relevance of animal

studies to human studies.

So I think there needs to be a discussion about this decreased capacity of immature animals to metabolically activate the OPs.

The second issue is that the report presents evidence in support of a role for A esterase and detoxification of certain OPs and in age-dependent sensitivity. But it doesn't discuss evidence that might be contrary to that view.

Out of the 30 or so OPs that we have, to my knowledge, there are only three that have been identified as being substrates in vitro for A esterase. Those are paraoxon, chlorpyrifos oxon and diazoxon (ph).

Over the past 5 or 10 years, there have been a number of studies based largely on kinetic analyses that have questioned roles, the role of A esterase in the detoxification of these three compounds in vivo.

Essentially, there is some evidence to indicate that these reactions are not very

favorable kinetically.

In addition, with the development of a knockout mouse by Clem Furlong, A esterase knockout mouse, he has reported that paraoxon -- in the knockout mice, there is no altered sensitivity for paraoxon. So we know that A esterase does not place an important role in the detoxification of paraoxon, which is the oxygen analog from parathion.

While Furlong's group has reported that the knockout mice do have an increased sensitivity towards chlorpyrifos oxon and para -- I'm sorry, diazoxon, and that's included in this report, Furlong has also reported that there is only a slight increase in the sensitivity of the knockout mice when the parent compound is given, which would be chlorpyrifos and diazinon.

And even then, it is only at fairly high doses of chlorpyrifos and diazinon.

So I think that that suggests that there may not be an important role for A esterase in the detoxification of chlorpyrifos oxon or diazoxon in

the knockout mice when the parent compound is administered, the chlorpyrifos or the diazinon.

So I think there needs to be some discussion of that.

And the third issue, in looking at table 2, the document states that the temporal pattern of A esterase and carboxylesterase activities correlate reasonably well with studies on OP sensitivity.

But the report doesn't discuss the possible exception to this correlation, which I mentioned yesterday, which is methyl parathion.

Methylparaoxon is not a substrate for A esterase. And according to table 2, it has limited interaction with carboxylesterase. Therefore, we should expect limited age-dependent sensitivity if we buy into the role of A esterase and carboxylesterase in age-dependent sensitivity. But with methyl parathion, it's age-dependent sensitivity, according to what is reported in table 1.

It is almost the same as that of

chlorpyrifos following acute exposure. And it is age-dependent toxicity after repeated administration. Probably even exceeds that of chlorpyrifos.

So I think these observations could suggest involvement of other factors in age-dependent sensitivity at least for methyl parathion. And I think that a discussion of that needs to be included in the document.

DR. ROBERTS: Thank you, Dr. Sultatos.

Dr. Pope.

DR. POPE: Yes, I have some of the same comments as Dr. Sultatos regarding the esterases and their role in OP toxicity.

One thing about most -- as far as I know, all the studies evaluating carboxylesterase -- many of the studies evaluating this esterase is an age-related sensitivity. There are correlation studies evaluating the inherent activity at a certain age group with its acute sensitivity to the pesticide. And there are no mechanistic studies really out there.

The paraoxonase activity is highly-correlated with age-related sensitivity, but paraoxonase appears to have no real role in parathion toxicity, for example.

The report mentions some toxicodynamic factors that may be important, such as differential receptor modulations, and also mentions the feedback inhibition of the presynaptic regulation of acetylcholine release, which I personally think is important in higher sensitivity in younger animals.

But that's going to be only important with when you are evaluating sensitivity at really high exposures.

I think roughly speaking the report does an adequate job of describing the information pertaining to differences and sensitivity based on cholinesterase inhibition.

DR. ROBERTS: Thank you, Dr. Pope.

Dr. Brimijoin?

DR. BRIMIJOIN: I really don't have much to add. I think Dr. Sultatos did an excellent

job. But what I'm hearing is that he has some very specific suggestions about some additional information, different points should be raised, should be incorporated in the document. And undoubtedly, we'll be able to capture that in the report.

But with those qualifications, I would agree that we're sort of close or on track here.

DR. ROBERTS: Just to throw in my comment, I think as a follow up to some questions and comments I think that Dr. Lambert made yesterday, I think there is -- probably the section on developmental aspects of P450 could be beefed up a little bit. There is a fair amount of information on P450 isoforms and at what points they come on line.

And if that could be tied with what information is available about those various P 450 forms in terms of bioactivation or detoxification of these compounds, that might be useful.

Any other comments or suggestions?

Dr. Hattis.

DR. HATTIS: I just want to apologize. I read most of my answer to this question in the previous discussion, and I'm sorry to have confused people.

But essentially, the only thing I have really to add here is that the relative importance of different activating and inactivating systems depends on the dosimeter that you think is causally relevant to the behavioral effects.

And one at least needs to discuss the different implications of different reasonable hypotheses about that.

DR. ROBERTS: Any other suggestions from panel members in response to 2.1?

All right. Perhaps then we should try and tackle 2.2 before a break, which would keep us on schedule.

DR. DELLARCO: Please comment on the timing of administration, in other words, the developmental stages treated, and the differential found between adults and the young animal.

DR. ROBERTS: Sort of an open-ended question.

Dr. Pope, do you want to tackle that one?

DR. POPE: Well, obviously, the timing of exposures is critically important if you are going to evaluate age-related differences in sensitivity.

The report describes a number of studies, some with prenatal, some with postnatal, some with combined prenatal and postnatal exposures.

Based on cholinesterase inhibition, the studies utilizing exclusively prenatal dosing appear to me to consistently report equal or lesser effects in the developing organism than in the dam.

As indicated in the report, this may in some cases be due to the timing of biochemical measurements relative to the exposures. If you wait long enough, you are not going to see a whole lot of inhibition in the younger animals because

they are recovering faster while it may not really be an indicator of reduced sensitivity.

In essence, more extensive cholinesterase inhibition is often noted in young animals compared to adults to a number of OP toxicants, postnatal animals.

With acute relatively high exposures, a number of organophosphorus insecticides, for example, chlorpyrifos and methyl parathion are more toxic to young individuals based on acute sensitivity, lethality, cholinesterase inhibition.

The ability to recover just as in prenatal animals between exposures and tissues from postnatal animals is probably very important in this regard.

If acetylcholinesterase molecules are being synthesized faster in immature animals, they will recover faster following each cholinesterase inhibitor exposure.

Because of the relatively short maturation period in rodents, however, repeated dosing studies have the confound of a changing

baseline. In essence, the animal is becoming less sensitive to the pesticide throughout the dosing period.

Thus, lesser age-related differences in sensitivity with repeated compared to acute exposures may be due to both inherent differences in recovery potential and to decreased sensitivity as the dosing period progresses.

DR. ROBERTS: Dr. Brimijoin.

DR. BRIMIJOIN: Actually, I still couldn't tell, I thought a lot about this question, and I couldn't tell what you are asking or why you are asking it and how it is different from what we have already talked about.

So I think Dr. Pope has done a brave job of plowing forward with a response to a question whose purpose is obscure.

Would you like to clarify your purpose, and maybe we could give you a little bit more help?

DR. DELLARCO: I actually think Dr. Pope was on the mark in what we were trying to get at.

Because when we were looking at the animal studies, just the empirical observations, we drew certain conclusions about prenatal exposure and what we see in the fetal tissues versus maternal issues.

And what we were seeing in the postnatal direct dosing studies with respect to -- it appeared that as the young animal was maturing, that differential was disappearing.

We just wanted confirmation, did you agree with those conclusions.

DR. BRIMIJOIN: So basically, yes.

I wondered if you were asking for more specifically like, do we accept the idea that a 21-day rat is equivalent to a one-to-two-year-old human, which is a key question sort of lurking in the background.

Do we think that a -- the dosing, how to handle this window of time between the birth of the rat and weaning it.

Do we consider that equivalent to third trimester, and what kind of dosing regimen would

be appropriate.

And I guess -- we had a discussion about that yesterday. And I think we're all aware of a certain sense in which this lineman is correct, but the questions about -- actually, the limitations of the model when it comes to modeling the very last stages of human development -- I certainly agree with what Dr. Pope has just said.

Since I'm on the spot, I'll just raise one other question. Maybe this is the right time to throw it in, or perhaps it should have been tossed in at 2.1, which is: In looking at these differences, which I'm convinced are real, that there are some compounds that are showing a definite age-related sensitivity in your model, and we have had some nice data, mostly presented by Dr. Padilla, about possible mechanisms, at least possible mechanisms that would account for these differences, and one of the things that has emerged is a consistent theme that when you go from acute dosing to repeated dosing at the very youngest ages, there are some chemicals that

behave differently, that chemicals which on an acute dose are -- the newborn or the very young are much more sensitive, and on repeated dosing that tends to go away, the explanation being that, this is something easy for us to accept, the idea that there is more rapid replenishment by new synthesis. The brain is adding to its cholinesterase pool.

In looking at those data, though, at first I'm just completely convinced, that makes great sense. I think it basically does make sense. But there is a puzzle that I would like someone else to comment on, maybe Dr. Padilla.

If we have some chemicals which are showing heightened sensitivity in the very young on acute dosing, but when we do the repeated dosing model, that differential is sharply reduced.

And then we have chemicals like methamidophos which don't seem to show this age-related sensitivity in the acute dosing model.

We had something like maybe malathion as an example of case 1 and methamidophos as an example of case 2.

So with malathion or maybe chlorpyrifos where we see the age-related sensitivity sharply with the acute dose and it goes away with repeated dosing. Metamidophos, we don't see it in either case.

If we don't see it in the acute dosing, though, and there really is a much more rapid replenishment in the very young, why doesn't the age sensitivity reverse itself when you go from acute dosing to repeated dosing with a chemical like that?

So if there really is such, as I believe there is, dramatic resynthesis, why doesn't that give the young an advantage with a chemical that doesn't show the differential sensitivity in acute dosing?

DR. ROBERTS: Dr. Pope would like to respond, apparently.

DR. POPE: In a way, we had a paper from

1993 that looked at intermittent dose in the chlorpyrifos. We actually did see that.

If you spread the doses of chlorpyrifos out far enough, at the end, the adult is showing a lot more neurochemical changes.

DR. BRIMIJOIN: Do you have anything to add to add to that?

DR. PADILLA: I actually have not looked at the repeated methamidophos study. So I don't know what the interval was. I don't know when they did the cholinesterase inhibition. So I really can't report on it.

But you are right. If everything else was equal, it seems like you might be able to see that sort of less sensitivity in the young after repeated dosing.

DR. BRIMIJOIN: Just having added this confusion, I'll just come back and say I basically agree with what Dr. Pope has said.

DR. ROBERTS: I was just looking at Table 1 in the document. The acute was done at PND 17, whereas in some of the other ones it was

done -- and of course, there was no difference, but some of the other ones were done at PND 11, acutely, and they did see a difference.

We're not necessarily having an equal basis of comparison, unfortunately, from the data set.

My impression, again, this is to emphasize something that Dr. Pope said, the problem with the model is that the development proceeds so rapidly that you can't repeat a dose at different stages.

Because to repeat a dose, you move through these developmental stages. And I think that makes it very difficult to try and get quantitative estimates of sensitivity at varying stages. Because to do any kind of a repeated dose, which I think we all agree is perhaps more relevant, you are spanning developmental stages.

So ultimately, you are only capturing, perhaps, what is relevant at the end.

What do you think about that, Dr. Padilla?

DR. PADILLA: There is also the aspect of how much each dose in each age carries over to the next day.

And if methamidophos is one of these compounds that the effects are really gone in both the adult and the pup by the next day, then what you are measuring at the end of the repeated dose, of course, is just the result of the last dose and not the cumulative effect.

That's the other factor that you have to factor into that.

DR. ELDEFRAWI: I thought we were looking at cumulative risk assessment. That means it should apply to all the OPs in use. Am I correct or am I wrong?

If some of them are affected by repeated dose and some are not, the organophosphate insecticides.

DR. ROBERTS: I don't know. Does someone want to respond to that?

Dr. Dellarco.

DR. DELLARCO: I'm trying to understand

what the question is. Could you restate the question?

DR. ELDEFRAWI: The repeated exposure versus an acute exposure or whatever for certain organophosphates but not others, they have different effects.

DR. DELLARCO: You are saying that for some of these OPs we can only see this increased sensitivity only after an acute and not repeated. In some of them we see after both acute and repeated. So how does that play a role in the cumulative.

DR. ELDEFRAWI: Yes.

DR. DELLARCO: When we look at exposure, we're doing daily estimates and we're also looking at exposure over a 7-day rolling average too.

It is kind of difficult for us to make a linear extrapolation into our exposure analysis from just these studies.

And the way that we're looking at acute and repeated is more with respect to developmental stages that were exposed and their sensitivity.

That's the point we're trying to make.

It appears somewhat as an animal matures, this seems to be going away.

DR. ELDEFRAWI: Could the toxicity be due to inhibition of acetylcholinesterases or are there other targets that are causing these symptoms.

Because if it's only some of the OPs, then it doesn't apply to all the organophosphate anticholinesterases. That's what I'm trying to --

DR. DELLARCO: You are saying this may be a characteristic that's not particularly shared among all these OPs?

DR. ELDEFRAWI: Shared amongst all -- I understand it is not.

DR. DELLARCO: Yes.

DR. BRIMIJOIN: Dr. Eldefrawi, I think maybe we're -- we're not talking about different mechanisms of action or things that would be outside the common mechanism.

We're talking about just differences in the life-span, the rates of metabolism, the depot

effects and other things, which will vary from one chemical to the next.

And the EPA has factored these things in to its regulatory scheme from the data base. So it shows how effects do build up or don't build up.

You can have 100 drugs that act by an identical mechanism, and each one of them will have its own unique pharmacokinetics and metabolism rates.

DR. ROBERTS: Does anyone else on the panel have anything to add to Dr. Pope's response to this question?

Dr. Harry.

DR. HARRY: I think your comment about this being a broad question that would be open for a lot of comments back on it is true.

And the one that was coming to mind, as I was hearing the discussions over there and also reading through the document on the changes that happened, and again, I'm sorry, I wasn't here yesterday, so I haven't looked there, is this

potentially raising a question of do you have the optimal design for exposure in your DNT testing that you have out there?

DR. DELLARCO: No. It really wasn't getting -- what it was trying to get at is you look at your animal studies. That's what you have. You don't have human studies.

But at some point in the assessment when you get to the characterization, you are going to need to make some extrapolations or predictions about children. And in our cumulative assessment, as I showed yesterday, we have different age groups that we're looking at.

So we just want to know to what extent can we draw conclusions about the sensitivity of different children's age groups in our cumulative assessment like the less than one year and infants versus the one to two year olds and so forth just based on what animal data we have that has looked at administration of these OPs to different developmental stages.

DR. ROBERTS: With that explanation, are

there any other comments we want to make on 2.2 before we go to break?

DR. HATTIS: I guess we'll just notice that we're going to talk about the enzyme development in children versus humans in another question.

DR. ROBERTS: That's correct.

And Dr. Pope, we may want to preface your comments with sort of a brief statement of what we understood the question to be, and then respond, because it is kind of a broad and open-ended thing.

If there are no other comments in response to this particular question, let's go ahead and take a break. Let's reconvene at 10:45. And we'll take up question 2.3.

(Thereupon, a brief recess was taken.)

DR. ROBERTS: Dr. Dellarco, could we proceed with question 2.3.

DR. DELLARCO: We're going to move to question 2.3.

Please comment on the extent to which

comparative cholinesterase data on six OP pesticides, chlorpyrifos, diazinon, dimethoate, methamidophos, malathion, methyl parathion, may represent a reasonable subset of different structural and pharmacokinetic characteristics of the cumulative group of OP pesticides to define an upper bound on the differential sensitivity that may be expected at different life stages of the immature animal.

DR. ROBERTS: Dr. Sultatos, what do you think? Is this a reasonably representative data set?

DR. SULTATOS: Well, the document suggests that the age-related changes in sensitivity to certain OPs is largely a function of pharmacokinetic factors. And I think I probably agree with that.

So to me, the answer to this question or to answer it, you have to consider whether or not the pharmacokinetic characteristics of the remaining members of the cumulative assessment group are sufficiently different from the six

indicated in the document so as to lead to juvenile, adult differential toxicity greater than three.

And it seems to me that based on the lack of information in the open literature regarding the pharmacokinetic characteristics of the remaining pesticides, specifically, with regards to their metabolism and volumes of distribution, I have to conclude that there is not enough information available to know whether or not the six insecticides indicated in the document are representative pharmacokinetically of the cumulative group.

So consequently, I don't think it can be concluded that those six OPs can serve as an upper bound for the possible different age-dependent sensitivity of other OPs.

DR. ROBERTS: Dr. Reed, what do you think?

DR. REED: I pretty much agree with what was said, but since I have written something out, I might as well read it to you.

The current available data on direct postnatal exposure, six OP pesticides, shed some light to the potential differential sensitivity of OPs during stages of development.

The agency is to be commended for the extensive effort in addressing these rather complicated issues.

However, the complex interplay of many factors, pharmacokinetics, but also pharmacodynamics, that are chemical and (inaudible) age specific that leads up to the inhibition of brain cholinesterase inhibition will give substantial uncertainty for predicting the upper bound of the differential sensitivity for all of the OP and their evaluation.

It is understood that the age sensitivity issue is somewhat important, especially for azinphos methyl, since the agency's presentation showed that azinphos methyl has 27 percent contribution to the food exposure of one to two years old. And I think that's sort of -- part of the reason that the question was phrased

making sort of comparison or mention of azinphos methyl and malathion.

Well, specific to the relationship between the two, azinphos methyl and malathion, the impossibility to predict the sensitivity pattern based on being in the same chemical subgroup is obvious and not necessarily limited to the age-related sensitivity issue of brain cholinesterase inhibition.

The improbability to extrapolate between OPs of the same subgroup can be illustrated merely among the adult female rats without the age factor.

A simple question is what considerations would predict the magnitude of more than three hundredfold difference of the two phosphoryl dithioates (ph).

Based on the agency's final cumulative OP risk assessment in June 11th, 2002, the relative potency factor is 0.1 for azinphos methyl and 0.0003 for malathion.

I looked at another phosphoryl

dithioate, methatathion (ph) that has a relative potency factor of 0.32. And there is a threefold.

So now we have, just based on the relative potency factor and brain cholinesterase in female rats, we have such a spread in differences in potency. And I look at that, and I decided I really cannot make an upper bound decision putting the age factor into it.

And I also make the observation that in another situation where I look at the impossibility of extrapolating (ph) the sensitivity pattern of brain cholinesterase inhibition between two chemicals just within the adult female rats, the chemicals that are metabolic activation pairs like acephate and methamidophos, and there is a more than tenfold difference in relative potency, again, this does not have age factor in it.

For these two chemical metabolic activation pairs, for these two chemicals, with the rich database available for methamidophos, the agency's document say that it's not possible to

determine whether acephate would show comparable responses in adult and young rats.

And so I felt that we're going through the same path as I personally have taken when I was working on methyl parathion. And even now our group in California is going through cholinesterase policy rediscussion or updating many of these issues, that we look at so many pharmacokinetic parameters, and I look at the polymorphism of any enzyme that I can think of, important enzymes for metabolism, and I came up empty in terms of using that to quantify the interindividual differences or age differences in any of these.

So I came to the same conclusion, too, with the agency that I decided to come back and just look at the how many fold, quantitative, how many fold difference is based on toxicity outcome. And that's where I think the agency's threefold came from, one to threefold.

My comment on that is that there is a place for that kind of assessment, but I think if

we are going to come up with that threefold from that type of comparison, then, as I mentioned yesterday, I think benchmark dose is important, and one of the data set, I believe, would come up to be fourfold instead of threefold.

So my conclusion is that I think threefold is, just based on that type of analysis, would not be sufficient to identify an upper bound of uncertainty factor that the agency is considering.

But I do have another issue I think is fairly important. I would not know where to place it, but since the FQPA uncertainty factor also addressed, as the agency interpreted, addressed the exposure, I thought it is interesting, and mostly in the context of what had been brought up as comments at many of these SAP meetings, I kept hearing people saying, the 99.9 percentile of exposure is really unreasonable and cannot be substantiated.

DR. ROBERTS: Dr. Reed, are you starting to get sort of into an exposure issue as opposed

to --

DR. REED: In terms of uncertainty factor overall.

DR. ROBERTS: Right. But can we come back to that point maybe later on when we talk about --

DR. REED: Yes.

DR. ROBERTS: You will have the opportunity to broach that issue then.

I gather, then, from your comments that you also do not think that the six necessarily captures the upper bound?

DR. REED: Right.

DR. ROBERTS: Great. Thank you.

Dr. McClain?

DR. MCCLAIN: This is a difficult question. And this is where the uncertainty factor comes in, is on this particular judgment. So it is a matter, I think, of looking about how certain or uncertain we are, but this is basically where the uncertainty factor is introduced.

And I think when you take a look at this

question, there is a couple ways that can be interpreted.

First is the question asked, can we predict the toxicity from the six OPs that we have data for. Or is it a question of predicting the degree of enzyme inhibition that may occur or the differential enzyme inhibition that may occur after direct dosing of the adults and the juvenile animals.

I think with respect to the first interpretation, it is certainly not possible to predict the toxicity of the chemical based on the toxicity of another chemical. One could only make some very generalized conclusions.

But what is being asked here is more limited. And that is, can EPA define the relative range of enzyme inhibition based on the amount of information that they currently have.

And I think you need to consider a couple things here. First, there is no inherent difference in the sensitivity of the cholinesterase enzymes between the young and

adult. And its binding characteristics, they are the same.

Second, the difference between inhibition of the cholinesterase between newborns, pups and adult animals is primarily due to two factors as we have discussed here, one of which is the rate of enzyme regeneration, and the other is the rate of detoxification by the various enzymes that are present, the esterases and the cytochrome P 450s.

And we'll be discussing some of the enzyme situations a little later on. And this certainly is an area where the information is deficient because really the detoxification enzymes seem to drive the differences with age more so than any other factor.

Now, these factors are, the enzyme, the rate of detoxification, the rate of regeneration of the enzyme, these, of course, are going to be the same with respect to any one of the organophosphates that you test.

And the main difference, then, between

compounds is going to be the relative rate at detoxification, which certainly could differ and does differ between the compounds. But in general, the six OPs for which data are available for cholinesterase inhibition of young and adult animals indicate that they are qualitatively similar.

And for these compounds, the ratio of CHE inhibition of the adult as compared to the juvenile, in this case the pup rat, would have sensitivity which range in several cases from no difference at all up to a threefold difference. And I think this is where the uncertainty factor comes in. And I basically agree with the choice of the agency.

And I think the other thing that needs to be taken into account here, when you are dealing with the prediction and the uncertainty of this particular aspect, is that the one to threefold factors that we're dealing with are based on the direct dosing of the adult and the juvenile animals, which is an appropriate way to

get some sort of an assessment of the difference.

However, under realistic conditions of exposure, that is the treatment of the dam, the pregnant dam or the lactating dam, the inhibition of cholinesterase is invariably higher in the adult as compared to either the fetus or the neonatal or juvenile animal. And I think this needs to be taken into consideration.

I think in the human infant, the level of enzymes that detoxify the OPs will be near the adult levels, and we'll discuss this again in a little more detail later, but by six months of age they are generally metabolically competent. And this would be at the point in time where you would begin to have dietary consumption of pesticides.

And I think these types of differences observed between pups and humans when you consider the six months of age are probably going to be different. We use our models to predict, but there is limitations on doing that.

And I think overall the prediction of the range of enzymes inhibition is more limited

than the predictation of toxicity. And I think the uncertainty factor based on this is appropriate at this time.

But one of the things when you take a look at this data, and we'll discuss this a little bit more, too, when you look at the differential inhibition of the enzyme between the various age groups, I would raise a question, is this a matter of exposure or is this a matter of increased sensitivity. And I don't think the two are equatable.

But that's my comment.

DR. ROBERTS: So we have a difference of opinion. In your opinion, the data set is sufficient to establish an upper bound for sensitivity --

DR. MCCLAIN: Acknowledging that this is where the uncertainty factor should be.

DR. ROBERTS: Dr. Pope.

DR. POPE: Well, to me, there seems to be little data to support the conclusion that six compounds would represent 30 compounds, basically.

If all 30 OP pesticides had exactly the same mechanism of toxicity and not just a mechanism in common, there would probably be sufficient information on the six. However, that's not the case.

If 24 other OP toxicants have not been evaluated, there is probably a high degree of uncertainty that all those compounds are going to behave in the same way as the other six.

And thus, the comparative data for the six representative compounds may not adequately represent the other 24 compounds, and caution should be used in that assumption.

DR. ROBERTS: Thank you, Dr. Pope.

Let me, then, ask other members of the panel for their opinions on this.

Dr. Hattis, then Dr. Matsumura.

DR. HATTIS: Basically, I agree with the earlier speakers in saying that I'm in general uncomfortable with using a term like bound because it connotes a defined upper limit when we -- unless, in fact, we have some good reason to

believe that values above X are not possible.

I would rather have a distributional treatment. But the distributional treatment has to be preceded by some better definition of the relative potencies in the pups of various ages relative to the adults.

And the current treatment -- I have been told privately that EPA is working on better treatments of these data. But for the record, you can't estimate relative potency appropriately, I think, by taking a number like 89 percent in inhibition in the pups and directly dividing it by a 39 percent observed inhibition in the adults for the same dose because even if there were no residual cholinesterase activity, 100 percent inhibition, that calculation couldn't get you an answer more than about 2.5.

If you -- you can treat -- the ideal treatment in cases where you have enough dose levels to calculate ED 50 or to apply Woody Setzer's types of models in calculating ED 10, you should use those.

And I have no problem with using an ED 50 or an ED 10 depending upon what is possible.

Where you have only one dose point to work with, you still can apply a simplified version of the exponential model that is basically the original model that was suggested earlier.

And basically, if you do that for this particular case where you have 89 percent versus 39 percent just for illustration, instead of the two point threefold difference that is indicated by the straightforward calculation, you get approximately fivefold. So it does make some difference.

It makes more difference in that case than in some other cases. And I haven't a complete handle on all of the things in Table 1, but essentially all of those calculations need to be redone, and then you need to do some kind of distributional treatment to describe the data.

DR. ROBERTS: Your original comment was, though, that you did not --

DR. HATTIS: I don't want to speak in

terms of bounds --

DR. ROBERTS: You don't think it necessarily sets an upper bound?

DR. HATTIS: Right. I don't want to speak in terms of bounds. At best, with a good deal of work, one can define upper confidence limits for the observed data.

DR. ROBERTS: Dr. Matsumura and then Dr. Needleman.

DR. MATSUMURA: This question whether it's really -- reasonably representing all organophosphates, I'm not sure, because I have experience such as the fenitrothion, which makes such a huge difference between the parathion and the fenitrothion.

And when you follow that kind of logic, it took a long, long time to understand why those two are different. And I guess the G S A -- G S T is one of the big functions which was not really considered.

Actually, I like Dr. Padilla's experiments very much. That's a good way to go.

That's a good solid progress. But like D D B P, which is one of the topics, the exposure that you really wanted to study but did not, they are affected by G S T.

And the glutathione really affect many, many of those OP toxicities; and there is no question, particularly dimethyl type chemicals and those halogenated and, of course, the double-bonded chemicals such as the D D B P.

And it is not represented here. And I mentioned about the carboxylamidase, which is not covered here either.

Of course, we have to keep working. And you are doing a good job going to that direction.

With a few more additions, you may have reached that goal. But at this particular stage, I have to side with everybody, Dr. McClain, Dr. Reed and Dr. Pope, that it is not there yet. That's my opinion.

DR. ROBERTS: Dr. Needleman and then Dr. Portier.

DR. NEEDLEMAN: Just a short response to

Dr. McClain's statement, that the children's behavior is not a measure of their sensitivity.

It is true. Children live closer to the ground. They put their hands in their mouth more often. They have higher metabolic rates. They take in more water per kilo than adults. They eat more fruit than adults. That increases their risk. And that factor should be included in the risk analysis.

Not to do that is to put them at increased jeopardy.

DR. ROBERTS: Dr. Needleman, did you want to weigh in on this particular question, though, in terms of whether or not the subset represents a reasonable upper bound or --

DR. NEEDLEMAN: No. I think it is well-said, well-handled. I agree with Dr. Pope.

DR. ROBERTS: Dr. Portier?

DR. PORTIER: Yes and no. And I'm going to go straight to the statistical issue.

Under the assumption that there is a common distribution for sensitivities across

chemicals between the adult and the juvenile, then, in fact, with six observations in a population of 30 possible observations, six observations should be enough to get you the mean and the standard deviation with sufficient accuracy to estimate some range of possible values for the difference between sensitivities in juveniles and adults across an entire distribution of 30 compounds.

Regretfully, that's not what was done in this analysis. And in fact, the interpretation you are using in applying these factors to your analysis for the differences between juveniles and adults is in fact to do it on a chemical specific basis.

Hence, in order to be able to do that, you actually need the numbers for every single chemical, because you are not presuming a common distribution and so you are not presuming a common upper bound. And the only way to get at what you are asking is to do the individuals.

DR. ROBERTS: Dr. Brimijoin.

DR. BRIMIJOIN: I'm going to give it an unsophisticated response here. We have heard I think very intelligent and informed reactions of people comfortable with statistics and population distributions.

But I'm talking my gut feeling is that the answer is flat out no. It is a huge data gap. And I think in the case of the compounds, that we don't have this developmental data for at all. We should revert to, in fact, the default FQPA factor of 10.

DR. ROBERTS: Other opinions?

Dr. Lambert.

DR. LAMBERT: Would it be helpful to poll the committee on this question if there is a divergent --

DR. ROBERTS: I don't know that we need to poll the committee, but I think -- I certainly want to give everybody who has an opinion the opportunity to express it for the record.

DR. LAMBERT: No.

DR. ROBERTS: Thank you. Very

succinctly stated.

Dr. Hattis.

DR. HATTIS: I want to add one other thing for the record.

A particular challenge for the proposed distributional analysis comes from cases like malathion where there is no detectable cholinesterase inhibition in adult animals in some -- in the brain, I believe. But there is appreciable inhibition at comparable and lower doses in younger animals. I think that was pointed out in discussion at the public session.

Simply -- the temptation is simply to exclude those cases, but there is a problem with excluding them. Because excluding those analyses could risk biasing the analyses because you have excluded the very case where there is a suspicion that the difference between adults and pups could be big.

So some kind of truncated distributional analysis is in order. And good statisticians know how to do that.

DR. ROBERTS: Last call for folks to express an opinion.

Dr. Eldefrawi, were you signaling me?

DR. ELDEFRAWI: No.

DR. ROBERTS: I think the panel response on this is reasonably clear. So let's go ahead and proceed, then, to the next question, which is 3.1.

DR. DELLARCO: This is our last topic area. This concerns the relevance of the animal findings to children.

The first question is: Please comment on the maturation profile of A esterase and the uncertainties surrounding these data in young children. Because no human data are available on the maturation profile of carboxylesterases, please comment on what should be assumed in humans, especially children age one to two years, given the animal data and what science understands in general about detoxification maturation profiles.

DR. ROBERTS: Dr. Hattis, are you ready

to respond?

DR. HATTIS: Basically, we have done some research in this area, although nothing is directly applicable without modification to the A esterase or let alone the one that hasn't been measured. I thought I might put up for you some of the data.

The panel, I think, has the paper that has this table in it. But basically, the thrust of the observations -- this is results from an analysis of a data base of pharmaceutical data, and it's basically observations of half-lives of about 30 odd different drugs.

This is some individual data. There should be a table that is in one of the slides. Again, even this slide is not easy to read.

But essentially, these are, essentially, from the overall regression analysis for a total of 41 different drugs for 135 different data groups.

Essentially, what we find is that premature neonates are about fourfold on average

-- or geometric mean, I should say, larger.

These are sort of one standard error limits on the mean on that typical result. Longer in half-life than adults.

That difference comes down to about twofold for full term neonates and ages up to about 2 months. By two to six months of age, the difference is no longer statistically detectable in general. By the time you get to six months to two years, the typical case is that the half-lives are somewhat shorter.

And thereafter, you have pretty close correspondence on average to adult levels.

The same basic pattern happens -- there was another slide that was like that that may not have gotten saved that shows a finer breakdown by different pathways.

In any event, this general pattern is similar to the hypothesized pattern from the limited data that we have for A esterase.

It doesn't guaranty that this pattern is going to be seen for the unknown metabolic routes,

but I think it is the reasonable best case. So basically, under this kind of thing you expect some increase pharmacokinetic sensitivity for very young infants under six months -- between six months and two years, which is about the period that was inquired specifically in the question.

You don't expect much enhanced sensitivity to increased concentrations of the parent chemical.

You could get some increase in generation of the active metabolite if those are produced by particular P 450 metabolic route.

So that's basically what comes out of our information. There is also some information that we have on individual values, and what you see is that you get individual values that exceed the -- even tenfold larger than mean adult values in some individuals early in life.

That tendency to have increased variability relative to the adults in half-lives does also tend to disappear by -- relatively early in childhood, two to six months of age

folks.

DR. ROBERTS: Thank you, Dr. Hattis. Does that conclude your response to this question?

DR. HATTIS: Right.

DR. ROBERTS: Dr. Lambert?

DR. LAMBERT: I took a pretty similar approach in trying to answer some of the issues on animal extrapolation to human that they've asked also.

And the agency should be commended for the document in their attempt to look at FQPA 10X for the OPs. The agency wishes for the SAP to comment on the metabolism of the OPs and in particular to A esterase. The overall premise is that OP neurotoxicities correlate with the capacity to decrease acetylcholinesterases.

Therefore, the expression and the turnover of the choline esterases may indicate the relative susceptibility of the developing human to the OPs.

The effect of OPs on the esterase appears to be dependent on the metabolism OPs to

the reactive metabolites of some of the OPs to oxons. Therefore, it would be informative to examine the entire pathway, and not just look at A esterase.

To begin with a general comment, a developing human is not equivalent metabolically to a rodent at any stage during development. To try to correlate any stage of a rodent's development and make it equivalent to a human's at any stage of development, for example, in the P 450, is just not -- there is no comparisons.

This is easily shown with the expression of cytochrome P 450s that are expressed in the human, and there are some P450s that are expressed in humans that aren't even expressed in the rodent. Those that are co-expressed in the rodent and the human have different metabolic profiles as far as developmental expression.

And most of this data is generated in the liver looking at the liver expression of these proteins and very little, if any, into the brain, where equal or greater would be anticipated.

Therefore, trying to draw any conclusions from an animal study metabolically to a human is very difficult.

A esterase may be a little less complex. But if you are looking at the entire metabolism of the OPs, that is going to be very difficult to come up with any reasonable comparisons that is accurate and similar.

The OPs are essentially -- some of them are initially metabolized by the P 450s to oxon metabolites. It appears the P 450s that are involved are primarily the 3a family and possibly 2D6. The family three enzymes' overall activity is generally thought to be increased during the newborn period, infancy and early childhood stage of life.

Family three development is primarily composed of in the human P 450 3 A4 and 3A7. The 3A7 is the fetal form of family three, which is not expressed at all in the rodent and as expressed, if at all, in very low concentrations in the adult.

And we essentially don't know anything about the ability of the human P 4503 family to metabolize these in vivo.

And particularly, looking at the fetal forms of the family, we have no data that I'm aware of, these findings are somewhat substrate dependent in the family three. And again, their ability to metabolize OPs during development is not available. That data is not available.

But the fact that these enzymes are activating some of the OPs to active metabolites are higher in the newborn and during early development, it would indicate that they may be putting the child at higher risk, the fetus, infant and early childhood.

In regards to cytochrome, P4502D6's expression is decreased, almost nonexistent in the newborn's liver, and then approaches adult levels within a few weeks of life.

The expression of these enzymes in the human brain during development has not yet been extensively studied, but it would be important to

look at.

In regards to A esterase animal data, there is only data in the serum and not any data as related in the human, and there is no data looking at A esterase activity in the human liver or brain.

There are no data about the maturation profiles of carboxylase in the human.

From the studies reported in the document, it appears that A esterase in the serum in both human child and animal are not expressed in early development, but develops to the adult level by one or two years of age according to what is given to us in the document.

This would again indicate that a child is going to make some of the oxons at a higher level, have active metabolites. And decreased ability to deactivate would be a concern and put the child at risk.

There are critical lack of data in regards to the human that prohibit accurate assessment of these pathways in the human. The

capacity of the P450s in the human liver and brain are not known. In particular, the capacity of 3a7. Also, the expression of A esterases and carboxylesterase in the human are not known.

DR. ROBERTS: Dr. McClain.

DR. MCCLAIN: I think this, as I mentioned before, is a particularly critical issue, is the detoxification enzymes for the OPs and their development both in the animals and humans since this seems to relate to -- probably would be the most important factor in the differential inhibition.

I did go back to this section on this question and read some of the papers that are referenced here. And of course, this question is specifically addressing the issue of the A esterases.

And the one paper here that did have data on the human developmental aspects, the Augustton and Barr paper essentially show that at birth in humans the enzyme activity is about 20 percent of the human adult.

And as you get to about six months of age, these are up around 70, 75 percent. And it would be consistent with Dr. Hattis's information that he showed that the clearance was about equivalent at about six months of age. So by six months of age, they would be, you know, close to the adults.

And the other question was the development of the carboxyesterase. There is no data available for that with respect to the development in human. However, in the literature that we were provided, there are a number of esterases. And they generally show a rather rapid increase after birth up to six months of age.

It is likely that the carboxyesterases would follow a pattern similar to the others.

DR. ROBERTS: Thank you.

Dr. Pope.

DR. POPE: Well, the carboxylesterases and the A esterases have been shown to be important in the detoxification of some OP toxicants, and may contribute to age-related

differences in sensitivity.

However, some studies suggest that other metabolic factors may also be important contributors to age-related sensitivity. The entire spectrum of activation, detoxification of the OP toxicants should be evaluated in relative sensitivity.

Determination of activities of all processes in human tissues would be ideal, but difficult to obtain. While the relative contribution of blood and tissue detoxification could be estimated and is estimated in animal models, information is unknown in humans. Thus, this kind of constitutes an uncertainty in how young children may respond to OP toxicants based on relative metabolic processing.

Both carboxylesterase and A esterase activities increase during postnatal maturation in rodents. Some studies suggest that esterases also develop in humans during the first year of life. These studies focus exclusively on A esterase, however, and only in the blood. Thus, the

knowledge of carboxylesterase expression is absent in any tissues of rodent models, and expression of A esterase in other important detoxification tissues, like the liver, is also missing.

One could assume that liver esterases may also coincidentally develop along with the blood esterases, but there appears to be no direct evidence.

It seems reasonable to assume that by two years of age, liver and blood detoxifying esterases have developed to adult levels based on developmental profiles on experimental animals, but there is no information to confirm that.

Data in human should be collected, if possible, at least with blood carboxylesterases to limit this uncertainty.

DR. ROBERTS: Let me open it to other members of the panel for comments.

Seeing none, I would just like to comment or second Dr. Lambert's information. We have done a little bit of work in my laboratory on perinatal and prenatal metabolism comparing rats

and humans in terms of P450 and asteratic metabolism. Unfortunately, not with organophosphorus pesticides.

But with the compounds we were looking at, there was nothing alike between humans in utero and perinatal and rats.

So it is an issue. There may be more similarities as development proceeds to approximately the one to two year age range, which seems to be the focus, but earlier than that.

I think there is some real question marks about using information from rats to extrapolate to humans to the extent that -- when metabolism isn't a key aspect.

Any other comments or things people want to add to this?

Dr. Dellarco, was our response reasonably clear?

DR. DELLARCO: Yes.

DR. ROBERTS: Let's go ahead and take 3.2.

DR. DELLARCO: Please comment on the

extent to which the biological understanding of observed age-dependent sensitivity to cholinesterase inhibition in laboratory animal studies informs our understanding about the likelihood of similar effects occurring in children. In particular, what can be inferred from animal and human information regarding the potential for different age groups to show increased sensitivity if exposed to cholinesterase inhibiting pesticides.

Does the scientific evidence support the conclusion that infants and children are potentially more sensitivity to organophosphorus cholinesterase inhibitors.

DR. ROBERTS: Big question.

Dr. Brimijoin, what do you think?

DR. BRIMIJOIN: We're now getting to the point where the rubber really is meeting the road.

Actually, this is really a continuation of the other question. It really is about asking us to what extent we believe that the animal data we have available, the data we have available,

which are largely animal data, apply to infants and children.

And that means, first of all, whether we think the types of age-dependent sensitivity that we see in animals really occur in children, infants. Whether the kinds of mechanisms that have been suggested to explain the age-dependent sensitivity in animals apply to humans in general. And then I guess even more specifically, whether it is the same relative importance of all these variables.

And of course, when you are faced with so many things at once, the tendency is just to throw up your hands and say, how could we ever know.

And so, I'm not sure that I can really inform this debate. Certainly, not based on my own specific knowledge of the relevant metabolic and pharmacokinetic parameters here. But I would say, I would take a stab at this, I think it would be very hard to argue against the idea that the existence of age-dependent sensitivity as seen in

animals would not be reflected by something roughly similar in humans.

And so I consider that the scientific evidence that we have now certainly offers a strong presumption that infants and children are potentially more sensitive to OP cholinesterase inhibitors than adults are.

So what I consider to be the debatable questions are, first of all, what is the exact extent or magnitude of this age dependency. Is it in the roughly threefold range that we have been seeing for some compounds in rodent models? Is it twofold? Is it tenfold? Hard to say.

Second, I think we have to ask what are the exact ages at which these putative changes in sensitivity will occur in humans.

How do we line up or do we line up at all the different stages of human development with the various phases that have been identified in a rodent model.

So in particular, I guess a very critical question, much the agency has focused us

on, is the extent to which a one to two year old child which seems to be at special risk of exposure because of behavior patterns and such, how closely we can model that case with, let's say, a weanling rat.

A third question is whether the underlying mechanisms of this age-dependent sensitivity are not only similar in general, but similar in specific terms.

And we have heard from Dr. Lambert in particular how at least some of the metabolic effects, particularly those involving the P 450 system, we have to say flat out that they are not similar. There is different enzymes involved, different expression patterns, different substrate preferences and so forth.

So even if we conclude that these mechanisms are in general similar, we have to recognize that there could be important differences.

And looking for the general similarity, I think the existing data where we have data in

human and animal together do support the idea that there is some commonality, that there is a developmental profile in the maturation of the A esterase family in particular, which if not identical in human rodent is fairly similar.

So I think to that extent we bridge the species gap. We know much, much less about the carboxylesterases, or the B esterases as Dr. Pope has pointed out.

We can make a guess. If I were going to set up a hypothesis, my working hypothesis would be it will follow the same pattern. But it is striking how little we know about that particular and possibly important variable, a variable that might be especially important with some OPs and much less important with others.

Finally, the issue of enzyme synthesis and replacement about the extent to which fetuses, infants, human infants will parallel the developing rat in showing much higher rates of resynthesis of acetylcholinesterase. Again, we have no data and very unlikely to be able to get

such data any time soon, if ever.

So it is speculative, although, again, a working hypothesis would be that from everything we know about the metabolic rates in children in general, it would be a safe bet that there is at least some degree of differential.

Is it as large as in the rat? Is it even larger? Cannot say.

I recognize that the panel here has to take some position on this matter, even if it's a determined decision that it can't take a position.

More than that, the EPA doesn't even have that luxury. They have to take a definite position. So we have to make or recommend decisions in the absence of a complete data set.

So I, with some and typical academic misgivings and concerns, would come down with the idea that the agency's basic approach of this is sensible in the absence of more information with all the caveats that have been mentioned.

However, I think that instead of just wringing our hands about the absence of relevant

human data and saying how hard it is to get it, I think we should actually do something about this.

These data gaps should be closed to the extent possible. And there are at least two basic ways that they could be closed in a relatively short period of time.

One is a much more extensive application of in vitro assays with human blood along the lines that Dr. Padilla has been using in her rodent studies to identify the potential role of A and B esterases in determining sensitivity, E C 50 values for OPs, but not limited necessarily to that approach. So that's the right place to start.

And getting blood samples is a minimally invasive procedure. And to the extent we can learn things from studying actual human tissues such as that, accessible tissues, I think it behooves us, the scientific community and the agency, to push for that information under the broadest possible scale with all of the relevant compounds.

And secondly, I want to raise again the idea that surfaced yesterday that I think we shouldn't contend ourselves -- or it's a false dichotomy to say we don't trust the rodent as a model for humans and we can't inject these things willy-nilly into humans, especially children, so we're stuck. I don't think we are stuck. There are other primates out there.

Primate research is encumbered with ethical problems, but the kinds of experiments that would need to be done to establish maturational profiles of these key detoxifying enzymes, the kind of experiments that would need to be done to show that in a primate, preferably a higher primate, that there is or is not a more rapid recovery of inhibited enzyme.

It is not a horrendous experiment. It is not even a terminal experiment. You might not want to do it on children, but the monkeys will survive.

So I think there should be deliberate thought given to pushing to get the most relevant

animal data that we'll be more comfortable in extrapolating the human case.

Those are my preliminary remarks.

DR. ROBERTS: Thank you, Dr. Brimijoin.

Dr. Lambert, do you have anything to add?

DR. LAMBERT: Let me finish up with the line he was going, and then I'll go back into my original.

It is kind of like in the -- I would agree with everything that Dr. Brimijoin stated.

As far as looking at kids, it is kind of like in the FDA issues with use of anti-hyperous and other drugs that are used in children that have never been adequately tested in children.

Some of the experiment in those drugs are going on, but we're not looking at kids to determine are we doing harm or benefit in the children getting those drugs and what are the optimal and safe use of those drugs.

And similar, the experiments, essentially, when you expose a general population

to a chemical, the experiment is on. And what we need to do is identify methods in ways to try and determine the outcome in the general population.

And yesterday, we talked a little about the exposure. In next month's epidemiology, there are a whole bunch of abstracts on kids' exposure to organophosphates in the July 2002 issue.

Some suggesting that kids in the peripheral -- in the rural, some around the farms are exposed to higher levels and some that aren't. There is dichotomy of information.

But in general, my comments are, the scientific data does support the conclusion that infants and children are potentially more sensitive to organophosphorus cholinesterase inhibitors.

The animal data is very helpful in exploring and understanding potential mechanisms of action.

In the field of toxicology, an almost universally-accepted concept is that extrapolation from the animal to the human for purposes of

quantitative risk assessment is very difficult and one of the most difficult areas of all toxicology extrapolation of data from the developing toxicology literature to the human.

And we can go back to thalidomide and we can go through all the usual examples of that.

The reason is that there are species and age-specific differences in P K P D and also end organ sensitivity, of course.

There are a few to no neurobehavioral studies that have been done in the human exposed to OPs during development. Although, we know we are.

In addition, the complexities and capacity of the human brain in comparison to the animal would imply that even if there are no acute or irreversible nerve behavior effects in an animal model, that the human may manifest neurobehavioral effects that cannot be determined or seen in the animal such as subtle learning disabilities.

Due to the total lack of data on looking

at the neurodevelopment of function of children with chronic high exposure to OPs, drawing any comparison from neurobehavioral studies in the animals is risky.

The human during development may be at greater risk due to enhanced metabolism OPs to oxon, altered sensitivities to the OPs and potential long-term and irreversible changes.

There is a clear need for additional studies. And this is all documented in the agency's report.

DR. ROBERTS: Thank you, Dr. Lambert.

Dr. McClain.

DR. MCCLAIN: I definitely think it is possible that humans could show some differences in sensitivity for enzyme inhibition with age as compared to rats. How this would actually compare, we don't know exactly. But I think whether or not this makes a difference is based on exposure. I think the bottom line of the issue that we're dealing with here has to do with exposure.

And I think what makes this cumulative risk assessment that EPA has done in the case of the OPs, especially well done, is that the exposure via the dietary route has been very well characterized for all age groups, probably a more comprehensive performance on this than they have ever done before.

It indicates that milk is not a significant source of OP in nursing infants. And for children, a comprehensive and data specific exposure assessment has been made with respect to dietary exposure.

And overall, the dietary exposures are very, very low in children. And this provides data, I think, with respect to the margin of safety by the dietary route, which is quite clear.

DR. ROBERTS: Dr. Reed.

DR. REED: About modeling and human response with animal studies, I totally agree with all the opinions being said in terms of in the absence of data that we just have to make such an assumption that there is a good likelihood that

humans, young ones, are going to be more sensitive as shown by the animal studies.

My only concern is quantitatively whether we could also assume that human young ones would have a threshold of 10 percent cholinesterase inhibition in the brain as sort of a benchmark.

And my concern came from the fact that a lot of neurobehavioral parameters, things that perhaps are a great more -- sort of greater importance to humans that learning ability or cognitive memory type of thing has not been tested.

Therefore, I cannot say whether going from the animal to studies quantitatively at the 10 percent level is sufficient.

DR. ROBERTS: Thank you.

Dr. Hattis.

DR. HATTIS: I just basically want to say that I support what Dr. Brimijoin said at the outset.

I think there is much more reason to

believe that there is purely pharmacokinetic extra sensitivity in the human neonate than at the somewhat later phases of development that where the exposures for dietary sources are higher.

For the neonate, however, it is very likely that there is some exposure by particularly inhalation routes that could still give enough to make the extra sensitivity in that initial period relevant to the cumulative assessment.

The water pathway as well is a possible source.

But the animal data do give us some extra reason to believe in pharmacokinetic sensitivity early on. It's a little bit more questionable on the basis for the period of maximum exposure.

DR. ROBERTS: The responses so far have all been fairly consistent. Let me ask the panel members if anyone else has a different opinion.

Dr. Needleman.

DR. NEEDLEMAN: As I sit here, the fable

of the blind-folded man and the elephant keeps manifesting itself before my eyes.

EPA has presented us with this elephant and blind-folded us and asked us to describe what it is. The two pediatricians here see the elephant as a child's brain. The toxicologists and molecular biologists see it as a collection of enzymes and proteins.

I think that we must focus on child development as the outcome of interest.

EPA has selected a single outcome, acetylcholinesterase, and is betting its money on that.

It employs it as a surrogate for other more direct measures closer to the outcomes of interest. That is, the function of the child, which is what we're interested in.

Now, we have this peripheral AChE levels. We don't know how they correspond to AChE at the critical site, the neuron, the neurite glia. And to assume the single measure of the peripheral enzyme may serve as a surrogate for

measures of disturbed anatomy or behavior, which is my interest, in the absence of studies of, once again, the degree of correlation between the AChE levels and the other outcomes, specificity sensitivity, predictive power positive and negative, is to introduce an unmeasured amount of uncertainty into the analysis.

And then to apply this exclusion, they will only consider other outcomes in the cumulative analysis as they relate to AChE inhibition -- is a mistake, I think.

AChE inhibition is not the mechanism of toxicity or the precursor of antitoxicity. It is a measure of toxicity. And until it is documented according to some of the criteria I suggested and probably others, it is a risky business.

Let me talk a minute about exposure prevalences --

DR. ROBERTS: I want to focus on this particular question and then when we finish after we get done with the last one, I think we're going to open it up to more, for individuals to raise

points related to this. I just want to be sure.

The question here is does the scientific evidence support the conclusion that infants and children are potentially more sensitive to organophosphorus and cholinesterase inhibitors.

DR. NEEDLEMAN: I think I'm coming to that. I would be happy to wait, whichever you prefer.

DR. ROBERTS: You know what is on your mind more than I do, but again, I want to focus the response to this question now. And if there are other issues related to this, but not directly addressing this, you will have the opportunity to make that.

DR. NEEDLEMAN: Let me go ahead. If you think I'm wrong, you will know.

I think there are factors which condition the way we examine this that important to make visible and bring up for discussion.

One is exposure. The OPP discussion of exposures is incomplete. There are important epidemiologic data on rates of exposure in the

literature. And they are not cited in the document.

Larry Needum (ph) and the people at CDC measured 12 analytes in 1,000 subjects in the 1984 NHANNES study. 82 percent tested positive for chlorpyrifos.

In Cienna, Italy, Apria tested six alkylphosphate (ph) analytes and found positive tests in over half of the children. That's a nonfarming, nonindustrial area.

In Minneapolis, Saint Paul, 90 children were tested. Positive detections were found in 98 percent of the children. Similar results were found in an urban sample on newborns at birth, meconium. 20 infants were studied by Robin Wyatt (ph) and the name is Barr. And they found that 19 out of 20, as I told you yesterday, had positive DEDP. And 20 out of 20 had positive DEDDP.

So those are very high exposure rates, and they cannot be shrugged off.

I want to talk about one particular issue in brain development that I think needs to

be thought of when we discuss the findings of behavioral alterations in rodents.

That's the issue of spearing of cortical function. It's a well-known phenomenon. That is, if you lesion a brain, there are recuperative powers that take place. And the animal may appear normal.

But if you later challenge the animal with other tasks, they would be deficient, because the cortex often comes in and takes over the function that was lesioned and then is no longer available for the later task.

It's a well-developed thing. It has been in the literature for 70 years. And I think it applies to the need for long-term studies of application of neurotoxicants to immature organisms or children.

I will close by saying we can learn something from history, too. 26 years ago in Crystal City, EPA convened under a court order a task force to write the criteria document for lead in children.

And after two days of vigorous discussion, the EPA presented -- the first pass of the EPA document said that five micrograms per deciliter was an acceptable level for lead in the air in the United States.

Now, five micrograms for cubic meter, excuse me, is about what Los Angeles was showing in a bad day. They wanted to say that that was safe for the entire country.

There were two days of very rigorous discussion, and the science advisory board told EPA not to revise the document, to tear it up and begin again, which they did. And they came back six months later. There was a second session. The document was improved, but still did not pass muster, and they were told to go back and come back with a better version. They did.

And the document called for a standard of 1.5 micrograms per cubic meeter, which became the standard for this country, and that was resulted in the removal of lead from gasoline. And in 1976, the mean blood less in this country

was 15. It is now less than 3.

And in this month's environmental health perspective, there is a kind of metric study from Centers for Disease Control which says that the monetized benefit to a one year cohort of children in this country, the children born in 1998, the monetized benefit for lowering their blood level over what it would have been had this not happened was between 118 and 300 billion dollars for that one cohort.

I think there is a historical lesson in that in terms of what science can produce in terms of threshold effect values and in terms of the potential benefits to society.

DR. ROBERTS: Does anyone else want to weigh in on 3.2 that we haven't heard from yet in terms of whether or not the evidence supports the conclusion that infants and children are potentially more sensitive to OPs?

DR. HATTIS: As you have rephrased it there, the sensitivity -- there is a distinction to be made between sensitivity to the

cholinesterase inhibition, which I identify as purely the pharmacokinetic and the pharmacodynamic part which is sensitivity to the effects that result from the cholinesterase inhibition, which I think by any standard there is just too little information on to be confident that we're -- that we know enough to say that the exposures that are consistent with that 10 percent effect level in the --

DR. ROBERTS: I think you are reading more into the question than was there.

Anyone else on this particular question?

Dr. Dellarco, were the responses reasonably clear?

DR. DELLARCO: We can move on to the last question or do you want to take a break?

DR. ROBERTS: Actually, I was going to propose that we take a break for lunch before we take on the last question.

Members of the panel have expressed interest after we finish the questions in perhaps commenting on areas related to the issues that may

not have been captured in the questions.

I have tried with varying degrees of success to forestall those comments until the end of the session. But I would like them to have the opportunity to do that. So I'm concerned that if we -- so there is, I think, a block of time that we still need to cover.

So let me suggest that we take a break for lunch for an hour, meet again at 1 o'clock. We'll deal with the last question and then have open discussions.

(Thereupon, a luncheon recess was taken.)

DR. ROBERTS: We have one more question.

DR. DELLARCO: This is our last question.

Please comment on the conclusions regarding the faster recovery in the young animal of acetylcholinesterase activity. Because there is no human information on the recovery of acetylcholinesterase in children compared to adults, please comment on the extent to which

recovery of acetylcholinesterase in children should be factored into conclusions regarding potential risk to children.

DR. ROBERTS: Dr. Elderfrawi --

VOICE: She is off chasing some wayward disk.

DR. ROBERTS: Dr. Harry, you are the representative among the discussants that is presenting. Are you ready to respond to this?

DR. HARRY: My question is do you want this short as I prepared as after everybody else or do you want me to prolong it until they get here?

DR. ROBERTS: You might need to stall just a little bit. Try not to get too expansive.

DR. HARRY: To directly address this question, it was asking a comment on the conclusions regarding that. And I guess we go back to the same thing in the fact that when I was reading through the document as well as looking at the slides this time, I'm not real sure that I saw exactly what conclusions you were drawing from

that.

Information was provided regarding what appears to be a faster recovery. However, there is little discussion regarding the dynamics of exactly how that happens. And I think we had mentioned that earlier, whether it is a dilution, what is the components behind the recovery.

And that that's actually rather important as trying to understand this biological impact of which to then a cross-over to say is this conservative enough now to take and to take into consideration when we're talking about children.

However, it reflects the data that you have on most of this. So it is not that anything was missed. I think it reflects the appropriate data.

Now having said that, I think what is interesting and as was mentioned a lot earlier by Steve in the last question was that the -- it is very difficult to assume that there would be something that would be happening in a rodent that

would not be an underlying component that would happen in higher mammals also at least to take into consideration.

The other thing that come out is the compensatory ability of the developing organism continues to show itself in a lot of different factions, and that has been examples today with the knockout animals as well as some genetic mutants.

We often see lots of things in there. And in order to take this to the human, you probably need to understand more about exactly what is driving that recovery. It was interesting, while there is a limited characteristic of what represents that recovery and there is an example of speculation of what it may mean or what may be driving it, very little data is available to you for the whole dynamics of that transmitter system as in what is truly involved, whether it is metabolism, whether it is the turnover, the enzyme activity, its receptor number, receptor binding and that type of thing.

And that information would be very helpful to you. I know you are looking for more information. I'm not telling you anything you don't already want.

But as far as the compensatory mechanisms which come into regard here, I would say that one should assume that such adaptive mechanisms will also be taking place in the human. And it is difficult to even say that you should discount any of that.

So while I would agree that there is no human information, you should take this into consideration when you are thinking about the humans. I have to honestly say I'm not real sure exactly what you are taking into consideration from the little bit of data that you have.

So it is a mindset for how you are looking at that information. But I think you are going on a body of scientific knowledge and all the other information that you have of trying to pull that out.

DR. ROBERTS: Thank you, Dr. Harry.

Dr. Eldefrawi, your comments on response to question 3.3?

Do you want to take a minute to get settled, or do you want me to ask someone else?

DR. ELDEFRAWI: My disks go away again today. I don't know.

DR. ROBERTS: Let me ask other members of the panel, then, on responses on question 3.3.

Dr. Brimijoin, do you have a response to question 3.3?

DR. BRIMIJOIN: Actually, I did prepare a response, but I think -- I basically included that response in my response to question 3.2, which is that I do think it is quite likely that there is an accelerated recovery in children, that this is something we have no direct data on in the human case.

This is something that is amenable to study in other animal models, including those that might be most relevant to the human case such as primates or even higher primates since it could be done as a blood base study involving injection of

OPs in measuring rates of return of plasma and erythrocyte cholinesterases carefully measured. I think that would be valuable.

That's really the essence of my view on this question.

DR. ROBERTS: Thanks. Let me, then, open it to the panel. Are there other members that would like to respond? Dr. Pope and then Dr. Hattis.

DR. POPE: Well, the recovery of cholinesterase activity, I think, can be an important determining factor in age-related sensitivity. It is, I think, only an important factor really when you have repeated dosing. It is a cumulative risk assessment that's based on primarily on repeated dosing. This should be an important factor to consider, that is to make the younger animal actually less sensitive than the adults.

One thing that doesn't come out, I think Dr. Hattis mentioned this before, is the functional status of the enzyme molecules that are

there. As I do when we treat animals, we will take tissues out and measure total cholinesterase activity. That doesn't really tell you where those enzymes are located in the animals' tissues and how they may be affecting neurotransmission.

And there have been several reports over the last few years that suggest that anticholinesterase may induce the synthesis of acetylcholinesterase and it may not be functional. So you may get a kind of a false perception of increased rapid recovery in the younger animal when it may not be really functional recovery.

DR. ROBERTS: That's a good point. Dr. Hattis and Dr. Eldefrawi.

DR. HATTIS: I think that's well and economically stated. I'm going to be less economical. Say it in ways that are maybe clear to different people.

The answer to the question depends upon -- again depends upon one's judgment about the casually relevant dosimetric relating cholinesterase inhibition.

If the most causally relevant dosimeter is peak levels of inhibition, then the relative faster rate of regeneration in younger animals doesn't matter much.

If it is in fact an AUC type measure integral of percent inhibition times time, then it matters a lot.

We don't know which is actually likely to be true based on the current analysis, which is one of the reasons for pursuing the issue of pharmacodynamic modeling a little bit more intensively as the data become available. It may be that the data are not really adequate for that. Maybe the in vitro data can shed light on that.

Some very tentative theoretical reasoning that might lead one to place somewhat greater initial weight on the peak dose hypothesis is based on this idea that the cholinesterase molecules associated with these synapse, mostly in the postsynaptic membrane, I gather, or attached to the postsynaptic membrane, are likely to have minimal exchange rates with molecules floating

free in the intercellular fluid or attached to other cells.

In this case, the apparent regeneration of whole brain cholinesterase following an acute acetylcholinesterase exposure -- anticholinesterase exposure, sorry, would be a function of both the establishment of new synapsis involving wholly new molecules and a likely slower rate of resynthesis of uninhibited AChE molecules in the cell body and then possibly somewhat slow transport of those new cholinesterase molecules down the long axon to the synapse.

In light of this, it is likely that after an acute inhibition event, a greater degree of inhibition will persist in preform synapses that would be expected from the recovery of whole brain acetylcholinesterase activity.

And I don't have a clue as to what the relative rates of that are, the resynthesis through generation of the synapse and maybe other places versus, as you said, the inhibition of the preexisting molecules.

But in any event, this has the potential to lead to a differential change in the activity of older neuro pathways relative to newer pathways either weakening or strengthening of things in ways whose effects I can't predict in advance.

DR. ROBERTS: Dr. Eldefrawi.

DR. ELDEFRAWI: I did ask my questions during the session, so I don't have anymore to ask. Thank you.

DR. ROBERTS: Thank you.

Other members of the panel who would like to respond to this particular question? Dr. Brimijoin.

DR. BRIMIJOIN: I wonder if I could ask Dr. Hattis for a little more clarification, just to make sure I understand, since we'll be writing this report together, and our this discussion might as well be heard by the audience.

I'm coming from a background where things like dosimetry and such terms are -- I have a tenuous grasp on them, but if I understand you correctly, when you are talking about dosimetrics

and dosimetry, you're talking about what measures of effect we're choosing to apply and how they might differ, how they might respond differently or show different things depending on the nature of the dosing itself, whether it was repeated or single. Is that right?

DR. HATTIS: That's almost right. But what I'm mainly focusing on is the cholinesterase inhibition as an intermediate parameter between the dosing schedule and the ultimate action in terms of changes in the structure and function.

And so what I'm talking about between peak dose and AUC is not necessarily in terms of the concentration or the actual amount of the anticholinesterase that is in the brain, but in terms of the inhibition.

DR. BRIMIJOIN: So in that case, it seems to me -- so you are raising the interesting question. It's a biological question about -- we're really focusing on the developing nervous system here. Is it worse to have a transient and relatively severe decline in acetylcholinesterase

activity or is it worse to have the same or possibly even greater area under the curve of a milder inhibition that is sustained for a long period of time, which I think is a question we can answer, as you astutely point out. That's a subject for further research.

But with that perspective, it seems to me that if we do focus on the repeated dosing instance as EPA has explicitly chosen to do as the most reasonable scenario in the actual field, it is that if we are talking about differences in rates of recovery, which in some cases may be significantly slower in the adults than in the newborns, then we're actually likely to have both things going on, namely, that although we might have a case where the bolus injection would have given comparable levels of inhibition, if we repeat that dose in an organism which has a slower recovery rate, the actual depth of the curve will be lower even if the individual ratchets in the curve are no larger.

DR. HATTIS: If you are talking about

the long-term accumulation of inhibition as the result of many doses over an extended period, then the rate of regeneration matters. That's right.

If are you talking about the peak or trough inhibition following a single event, then it matters less.

DR. ROBERTS: So what I'm hearing is that there is at least in principle the desirability of including that information, but how to include that information. I mean, how to include differential recovery is hampered by fundamental lack of information.

DR. HATTIS: Yes. You have to basically have a dynamic model of cholinesterase inhibition in the relevant brain and recovery.

And it is possible that there is enough information to do that, but it would most certainly be aided by additional dynamic modeling exercises -- maybe even some additional, you know, exercises in data collection, because it is possible that the neuroscientists have not been as interested in these modeling enterprises as

basically quantifying --

DR. BRIMIJOIN: As they should have been.

DR. HATTIS: I'm trying to say this very gently that sometimes biologists don't have the same orientation toward quantitative issues as some random risk assessors trying to look over their shoulder and use their results.

DR. ROBERTS: Any other comments in response to this question?

Dr. Bigbee and then Dr. Matsumura.

DR. BIGBEE: There is data in the adult. I don't believe in the young. And this is results from Mona Zurick's (ph) laboratory, that inhibition of the acetylcholinesterase leads to the expression of a novel transcript, a novel splice variant, which she calls the read through form. And this enzyme is active, but it is a soluble monomer.

If you were to look at total AChE recovery, you would be measuring this novel read through transcript. But it wouldn't be placed in

the membrane or at the synapse as precisely as the normal synaptic form.

That's shown in the adult. I don't think there is any data for young ones.

DR. ROBERTS: Thank you.

Dr. Matsumura.

DR. MATSUMURA: My position is similar to Dr. Harry. Yes, it happens. It is probably fundamental. And probably that may happen in the humans too, real young child, but it is interpretation.

If you think every compensatory or repair process is good for that animal, then we have a problem. We cannot make that kind of blanket statement just simply because those young animals can recover quicker so that's not a problem.

You cannot make that kind of a statement. So what I mean is that the distribution packaging -- lots of people assume that the recovery is due to just the quick synthesis. It may not. Proteins must be

phosphorylated, packaged right. It could be having splice variance.

There are many, many ways that the proteins could show the increase in functions for that time of duration. But it is not always that compensatory or repairing mechanisms good for the animals.

All I'm saying is that we cannot say always that the fact the young animals can recover quickly does not mean that it is always more poisons, problems disappear there.

DR. ROBERTS: Any other comments?

Dr. Dellarco, do you have any follow-up questions on this? Was our response on this reasonably clear?

DR. DELLARCO: Yes.

DR. ROBERTS: Great. Thank you. This concludes the responses by the panel to the questions posed to it.

Before we move on, I would like to point out that Dr. Portier had to leave over lunch. He was not able to participate in discussion of this

last question or subsequent discussions.

He did ask me, though, to communicate to the agency that despite his pointed comments earlier, he is in fact very pleased with the effort in the document that you folks have produced.

So I wanted to communicate that final message to you from Dr. Portier.

I had promised the panel the opportunity to make some perhaps more general comments. And let me say at the beginning that it is not my intent to open up the cumulative risk assessment in total to comments.

SAP has been consulted on numerous times about the cumulative risk assessment, including as recently as just a few months ago. So I think we should let -- our suggestions are on record. I think we should let them stand.

The topic for this particular session is the determination of an appropriate FQPA safety factor in evaluating sensitivity and susceptibility to the mechanism of toxicity.

And within that subject area, there are perhaps some comments that in the judgment of the panel might be useful for the agency that don't fall in the context of the specific questions.

So what I would like to do is to provide the panel with the opportunity to make those questions now. And I suspect it is going to impossible to avoid some sort of ping ponging around on different subjects, but I would like to the extent possible for us to focus on one subject and make whatever comments we're going to make and then move on.

Intuition tells me that one of the subjects that panel members might want to comment on is the scientific underpinnings regarding the specific choice for an FQPA safety factor made in the document that we reviewed.

In other words, did the data with what it offers and what -- its limitations support the choice made by the agency.

So I will at this time entertain comments from panel members on that subject if you

want to weigh in or if you have an opinion to express.

Dr. Brimijoin.

DR. BRIMIJOIN: This is a question. It might lead to a comment -- but since we still have the EPA representatives here, and Dr. Dellarco, for example, in particular, put her on the spot.

I mean, you have heard from the panel various levels of comfort and or discomfort with the proposal to in general apply a threefold safety factor, F Q P A factor into the RPF's or benchmark doses of certain compounds.

I guess you have heard from us that we think a tenfold safety factor is more appropriate for the compounds where you have no data at all.

I would like to ask a very practical question of you, which is whether you have done calculations that show what would be the ultimate impact on the viability of the, let's say, currently registered chemicals, if you went to a uniform FQPA factor of 10 as opposed to three, I would just like to have some sense about whether

we are skating the edge of something that makes an enormous difference in whether any chemicals can ever be used or -- you don't have to name chemicals and companies, but as to whether there will be a radical change in the landscape depending on whether you finally end up with factors of three or factors of 10.

Do you think you could answer that question?

DR. ROBERTS: Let me offer the agency the opportunity, since this doesn't relate to a particular scientific issue, but sort of the consequences of scientific decisions.

If you want to respond to that as a side bar rather than in this session, certainly that's okay with the chair.

DR. DELLARCO: We can only respond to that to a certain extent.

And based on the understanding of exposure to these OPs and their relative toxic potency, you would have the same contributors that we identified yesterday. They would still be the

major contributors.

DR. ROBERTS: Dr. Dellarco, I actually had a clarification. And it came from a comment that you made yesterday, and maybe I didn't understand.

By applying the factor in a sense sort of early in the calculations to the potency factor, then it really gets carried -- it really gets applied regardless of the age group. Is that true or does it get applied specifically for the margin of exposure for that age group such that it would not get applied for adults?

I guess it really just depends on where this gets plugged into the process, how it translates out through the calculations. That was just something I didn't understand.

DR. DELLARCO: We incorporated the 3X on the RPFs, and we did it across all age groups, even the adults, simply because the one-to-two-year-old age group is most highly exposed.

DR. ROBERTS: I'm sure it is more

convenient from a calculation standpoint to do it that way, but of course, it does distort a little bit the comparisons and the margins of exposure from different age groups. I just wanted to get that clarification.

DR. PERFETTI: You are absolutely right.

It does sort of distort the other age groups, but our feeling was is that we knew that the one to twos were the most highly exposed, and that all of the other exposures were within acceptable ranges.

So I guess we should have made clear, and I will make clear now, that the exposures for the other age groups are much exaggerated by about 1.2 overall.

DR. HATTIS: I'm not understanding that.

DR. PERFETTI: Because of the software and the way it runs, we could not selectively put the factors on the RPFs and then apply it only to one age group. We had to apply it to all of them.

And if you wanted to know what the actual exposures were, you would have to then go

back and hand calculate exposures for other age groups.

DR. HATTIS: You could do a post processing. This is the estimated exposure that is in raw milligram per kilogram equivalents of the standard chemical, and this is what you get if you apply various FQPA adjustments to different age groups.

It might be easier to do a post process.

DR. PERFETTI: Believe me. We thought about it.

DR. ROBERTS: Thanks. Any other comments. Dr. Reed?

DR. REED: Maybe I should ask sort of for a clarification first.

My understanding by reading the document is that the FQPA safety factor would apply based on your consideration of not only on the toxicological part of it, but also the exposure.

And so my earlier comment was within that context, in that the question was posed as is 3X enough considering the toxicological part of it

with an understanding that the exposure is extremely conservative or at least we don't have uncertainty in that sense that we know how to estimate.

And I think that is an important point to bring up, especially now that the panel is pretty much in agreement in terms of threefold not being sufficient to address the toxicological part of it.

Especially in that context, I think it is important to take a look at the exposure and be very sure that we don't have any underlying uncertainties that would come with it.

And my comment is it is a good practice and you have been doing this in expressing the exposure in a range with the different percentiles. But it was sometimes looked at as, okay, then one might have a choice of taking at the 95th or 99.9 and so forth and it depends on how we look at the data in the outcome.

What I did, and I think it would be of interest to you, what I did was to take what was

presented in that table with different age groups and different dietary exposure levels at different percentiles.

What I did was to take that number and assuming that all that exposure actually came from only one commodity and one pesticide, not one commodity, multiple pesticides or modical commodity, modical pesticides, which is quite cumulative risk assessment as well.

So as sort of putting meaning to number is what I was trying to get. I think it is a very important point so that the people would understand what does 95th mean outside of the consideration of statistics. Because if you do the statistical sort of consideration, you would say, well, 95th is probably more certain. And since we have all the real good data in there and 95th might be a more firm number and 99.9 might be pretty far out on the distribution.

So that's what I did. I took the exposure value and attributed that, all of it, to one chemical, and one commodity in this case --

because azinphos methyl has 27 percent contribution. I think you are more interested in looking at azinphos methyl because of the lack of data about young ones' sensitivity.

So I went back to the PDP data. We're making sure that we're not using something that is extremely unlikely as, say, tolerance, less than one percent chance.

I went back to 1999's PDP data. I looked at two commodities. One is azinphos methyl in apple. The single serving survey would have 76.2 percent of detect, so it's not an unlikely event in terms of being detected to have residue. And of course, there is a range of residue level.

What I did was to take the highest, which is 0.55 PPM for the single serving apple, and back calculate with that exposure level, and now you know the residue concentration. You assume a body weight for one to two years, 10 or 15 kilogram.

Then what I come up with is a consumption, a different percentiles of exposure

that you come up with a cumulative risk assessment.

For the 95th percentile, a child one to two years, so it would eat less than two ounces of apple, if you attribute all the exposure only come from one commodity, one pesticide, and so that 95th becomes not representative, in my mind, not representative of high end at all.

So you go up to 99th, 99.5 and 99.9. And I think it might be good for the agency to present sort of a meaning to the number in such a way so that a reader could understand what does he mean by 95th percentile exposure and what is 99.9 exposure.

What I did also with pear, for single serving pear you have 43.2 percent detect, which is, you know, again, not a rare event. By the way, I still eat apple and pear, and I haven't had any concern about that. So it was not about the commodity. Not about the pesticide.

You have a detection range. Pear, for single serving pear, you have actually higher

concentration than the apple.

So at the 95th percentile, if it is all attributed to pear and only coming from the exposure of azinphos methyl, it would amount to about one ounce of pear per day at the 95th.

So I don't think it is very quote, unquote conductio (ph) or capturing the high end at all.

It's sort of justifying for both taking a look at it, but also for making perhaps a risk management decision later on after the risk assessment to decide where you want to take the decision based on what percentile.

DR. ROBERTS: Dr. Eldefrawi.

DR. ELDEFRAWI: I was wondering, the pear or the apple, is it peeled or is it eaten with the skin?

DR. REED: Could someone comment on that with the P D P data on a single serving survey?

DR. PERFETTI: Actually, in the P D P data, the fruit is washed, lightly washed. So it would be with the skin. But in our software

program, the DEEM, there are provisions made for both peeled and unpeeled fruit.

DR. REED: I guess the difference between peeled and not pealed is really dependent on whether a chemical is systemic or not. If it's systemic, then peeling probably is not going to make any difference.

DR. ROBERTS: Right.

You did make mention, before I get to Dr. Hattis, who is next on the list, that the opinion of the committee is that threefold is not sufficient. And I don't know that we have established that, which is sort of the purpose for our discussion now. I just wanted to point that out.

Dr. Hattis.

DR. HATTIS: I think part of the argument on whether threefold is really plenty or tenfold should be retained goes to the sufficiency of the evidence for assuring safety. And part of that discussion, you know, relates to the claim, the perception that is created by these margin of

exposure numbers of the order of 100 or somewhat more or somewhat less.

I think it is worth remembering what the 100 was for and, to some extent, you know, what its limitations are. Because the one hundred is usually thought of as tenfold for between species differences and tenfold for among human differences.

The tenfold for between species differences, however, is based upon measuring dose in terms of milligrams per kilogram of intake in the animals.

And as it happens, that's not the most predictive dosimetric for toxicology in general for chronic effects.

For acute effects, it is in fact the best dosimetric for things like L D 50s. They scale across species more or less like that.

But for effects that take several doses to produce or internal levels, it turns out that pharmacokinetic processes, elimination processes tend to scale on average with body weight to the

three quarter power. And between rats and humans, that use is up about fourfold of that tenfold.

Secondly, so that you are typically -- there is only about two-and-a-half fold left or twofold left of conservatism in that interspecies factor once you take the average pharmacokinetic differences into account.

Then if you compare effective doses in humans with animals after making this correction of body weight to the three quarter -- taking the body weight to the -- you still get substantial variability from chemical to chemical in toxicologically equivalent doses. And this is based on a series of comparisons by Paul Price with anticancerations with not exactly the same endpoints in animals and people. But it's worth mentioning that for rat single species you get on average about, human potency, about .8, what you would predict on the body weight to the three quarter basis.

But the observed confidence limits around that, that is the -- is basically there is

a geometric standard deviation of about threefold that describes the distribution of equivalent animal and human doses.

So what that means is that where your best expected value is close to one, your 95th percentile is for human potency that would be about just a little less than fivefold more than the animal, the prediction of human potency that you would get from the animal based upon the body weight to the three quarter power scaling.

So essentially -- you shouldn't expect that that tenfold is in fact -- is going to be on balance, a little conservative, but it is -- it comprises much less than a 95th percentile of that particular distribution. So it has some conservatism built-in it, but not a great deal.

The tenfold for human interindividual variability I found from a database of observations may well not be doing the full job that people expected to be doing, that essentially the human interindividual variability from my limited data sets, which are generally not

including the full range of sensitivities, would not be sufficient, usually -- would not be sufficient to get you from a dose that is causing 10 percent incidence of effects to a dose that is causing 10 to the minus 5th incidence of effects a large fraction of the time.

It will most of the time, but again, it is not a lot of the time. And if we build in the fact that my interindividual variability observations don't include really a large number of effects that would be distinctive for early life exposures, then there is some argument for an additional safety factor for developmental type exposures that could be associated with developmental changes.

Going more explicitly to the legal language that Ruby was raising, I have to say that I don't think that a reasonable standard of "adequate" evidence is met on the pharmacodynamic side.

I think you could conceptually distinguish between the pharmacokinetic side and

the pharmacodynamic.

In the pharmacokinetic side, I think we have some insight that would lead to us suggest that -- if we have no pharmacokinetic information for the chemical and no pharmacodynamic information, then maybe you should be retaining the full tenfold safety factors.

Where you have some pharmacokinetic information, there is a possibility that you should make a lower adjustment in recognition of the fact that you have eliminated some of the uncertainty by the pharmacokinetic comparison. But we don't have very wonderful pharmacokinetic information in the humans.

In fact, for the very young humans, there is good reason to suppose that there is an extra fewfold prolongation of half-lives, at least for newborns and up through several months of age.

By the time you get to the age that you have been focusing on for the greatest exposures, I think it is quite right that we don't have very many examples of unusually prolonged half-lives in

that case.

That doesn't mean it couldn't happen. But we just don't have much observational data that supports that. I don't want to make an overall policy suggestion, but I do want to suggest that Ruby is right, that if you want to apply some understandable standard of adequacy of evidence on the pharmacodynamics side, as a general mater, I think that some considerable skepticism needs to be retained.

We have some, but rather limited, pharmacokinetic information, so that there is an argument that could be made that you have some evidence on that front, but whether it is entirely sufficient or whether the pharmacodynamic concern is sufficient that you want to retain the whole tenfold for pharmacodynamics is certainly a possibility.

DR. ROBERTS: Anyone else like to express an opinion on this issue?

Dr. Reed.

DR. REED: Could you just clarify. What

I think I'm looking at is that there are certain things that you can clarify more and get you out of that uncertain mode. And I think exposure, especially dietary exposure, is one.

If you could clarify what the exposure express, then you might be able to say, because I know so much of it, I don't have to include that in the uncertainty consideration.

DR. ROBERTS: Anyone else on this issue?

DR. LAMBERT: Are we taking it for granted that the panel feels that 10X is the appropriate or we're not going to discuss it?

DR. ROBERTS: I'm not taking that for granted.

VOICE: If you want to express an opinion, speak.

DR. LAMBERT: As far as I'm concerned with what Dale had stated, I think you can take into exposure the concepts, but I think what we have for kids right now on exposure is probably -- in the food chain, water and food is probably pretty good as far as we have been discussing a

couple times.

In inhalation and drift off of fields and things like that, that's a much different database, which I don't think there is adequacy at this point. But there may be in the very short term. But some of the initial abstracts that are coming out, at least in some of the studies, are suggesting that there is a significant higher exposure in those kids living in and around farms using these chemicals.

But if you just take what Dale had said as far as the pharmacokinetic and then put it into the formula, the dynamic aspects of potentially a more susceptible organ system in a child, particularly with potential of having long-term effects on the brain, I would think that due to the inadequacy of what we have in front of us and as we just stated today that we felt that much of the data was lacking and there was in some of the pharmacokinetic aspects that the 10X factor would still be in play.

DR. ROBERTS: Anyone else want to

venture an opinion on this? You are not compelled to do so. Just offering the opportunity.

DR. MATSUMURA: Just a clarification.

This particular discussion is not going to be a part of this answering session. Right? So it is more a free discussion rather than --

DR. ROBERTS: It would be covered under a comments section at the end of our report.

DR. MATSUMURA: I was thinking the perspectives. At least most of those are registered pesticides. It has been used for 20, 30 years. And of course under the FIFRA, most of those people, all of us are being exposed.

So my overall feeling is that organophosphates or phosphorous pesticides and carbamates, they are not that huge problems that something that we have seen like organochlorine and all those pesticides just simply because their actions are rather ephemeral, exception, delayed ataxia, all those, the chronic type, the organophosphates which have been eliminated, leptiphos (ph) and EPN and all those chemicals

have been already eliminated, and even the methyl parathion is gone.

So my feeling is that at least perspectives, I may go along with the agency's currently recommendation for this particular case with some reservations as expressed.

That's my feeling looking at the more comparative ways. I really do not see such a social disaster like the lead poisoning or mercury or those which stay in the body for long, long time like cadmium arsenic.

I don't see that. Metabolically, they are eliminated rather quickly. That's my feeling.

DR. ROBERTS: Thank you, Dr. Matsumura.

Last call.

DR. HATTIS: I don't see evidence of a wide spread disaster either, obviously. But I'm not sure we would know. I'm not sure anybody knew about lead, you know, at a comparable stage in the development of the issue.

And that was in the face of mean blood lead levels of the order of 19 or 20 or something

like that, that you perhaps can give that.

In any event, the policy choice was made by the Congress to a degree that said that unless we are pretty damn sure, we're supposed to retain this factor.

DR. MATSUMURA: My point is the persistence in the animal data. As the active form, how long those chemicals persist in the body. So what I can -- immediately, that's clearance, is not comparable to anything like PCBs or lead or mercury.

These are the ones which half-life is rather short. That's what I'm saying. Just overall feeling.

DR. HATTIS: It is quite right that the persistence is much less and that's a factor arguing for less concern than was in the case of either lead or the organochlorines.

On the other hand, there are these mechanisms that are at least possible whereby you have a transient change leading to long lasting effects.

DR. ROBERTS: Dr. Needleman.

DR. NEEDLEMAN: Can I pick up on what Dale said about lead, because I think the history is instructive.

When childhood lead poisoning was first reported, there was great skepticism that there was such a thing, that children could have lead poisoning was disputed.

Once it was accepted that, yes, kids could get lead poisoning, it was thought there were only two outcomes, you either died or you recovered completely with no residua.

Then it was accepted that there were long-term effects. Now we are talking about 1943. But in order to have long-term effects, you had to have signs of brain edema, vomiting, convulsions, stupor.

At that time the toxic dose was established at 60 micrograms per deciliter. Then it was shown in the 70s and 80s that children who had no visible symptoms but had elevated body burdens had lower IQ scores.

And the threshold for effect shifted downward to 30, 25. And then CDC and NAS said it was 10 or lower in 1980, I think.

Now there is data that shows that blood leads below 10 are associated with measurable deficits in IQ. And the reason for that is better outcome measures and better epidemiology.

There is a reciprocal relationship between the quality of the studies and the effective dose.

DR. ROBERTS: Dr. Harry.

DR. HARRY: Sorry. This is a quick comment on the history. And while it was appreciated, and I think we do remember that, we also have to realize that we're not starting from that same point. We are using those refined techniques now. We are looking for those subtle differences in animals as well as in the epidemiology study.

So I don't think any of us are going to forget the steps with the lead. And I'm not real sure that assuming that taking a 3X versus a 10X

factor is going to take us back to the times of not recognizing that there are risks, because we do have those refined methods that we're using across the board now.

DR. ROBERTS: Dr. Needleman I think would like to respond.

DR. NEEDLEMAN: I just have to dispute what you said about the quality of the outcome measures. I don't think we're applying the same specific measures of function, behavioral function.

DR. ROBERTS: Are there any other comments on this particular issue? Anyone else want to weigh in?

Mr. Lewis has suggested that I summarize our comments on this. And I'm reluctant to do so.

We did have some folks express the opinion with different explanations for why they thought an FQPA safety factor of 10X would be more appropriate. And we had one panel member express an opinion that the 3X was appropriate.

That essentially, I think, captures the

discussion so far, although, many of the panel members, maybe even numerically most of them, did not express an opinion on this issue.

Dr. Harry.

DR. HARRY: As a point of clarification, on each one of these compounds, you have an individual evaluation that you have done. Right?

How is this cumulative risk assessment going to influence an individual chemical's risk assessment?

MS. MULKEY: Let me try that. It is not really how it influences the risk assessment.

The individual chemical risk assessment does not, except to the extent that the same issues are relevant and they appear there, it does not adopt or borrow from this risk assessment.

But to draw a conclusion from whether the tolerances which are, of course, are all set on individual chemicals about whether they meet the statutory standard, the reasonable certainty of no harm standard, you have to have evaluated the individual chemical's risk assessment and

drawn your conclusions based on that.

And then the statute says you have to consider the cumulative risk associated with -- if that chemical is part of a group that has a chemical, a common mechanism.

So before a final determination can be made about whether a particular tolerance meets the standard, the reasonable certainty of no harm, you have to have considered the individual chemical risk assessment and considered the assessment of the cumulative risk from the class of compounds.

So the individual chemical risk assessment looked at the same data, was informed by the same underlying information, as well as a lot of other information. But it was not per se influenced by this risk assessment.

DR. HARRY: I was just wondering how this influenced that and also to bring back the fact that in each one of the individual ones you do look at all the behavioral outcomes, you look at everything that may happen there, adult and

developmental as you have them in.

And then this is sort of an extra component of information of how things might be additive to evaluate how do I now look at these things of how they may build up and work with each other.

DR. DELLARCO: Exactly.

DR. ROBERTS: Then let me now open it. Are there any other scientific issues related to whether and how to use information on the sensitivity of children and incorporate that into the cumulative risk assessment? Any comments on that area that individuals on the panel might want to make? This is sort of our last offer for comment.

DR. HARRY: Could you say that again?

DR. ROBERTS: Now moving beyond the issue of the specific FQPA safety factor, but, again, within this topic of how the agency should view and use data relevant to a determination of sensitivity of children and incorporating that information into the cumulative risk assessment,

are there any comments that people want to make that weren't covered previously in our response to the questions?

Dr. Pope.

DR. POPE: I would like to ask the EPA people if -- with the single compound risk assessments, are any of the compounds regulated on the basis of something besides cholinesterase inhibition?

DR. DELLARCO: Yes. Because all toxicities are considered. And typically in those assessments, they go for the sensitive endpoint. It may not necessarily be cholinesterase inhibition or cholinesterase inhibition in the brain.

MS. MULKEY: In most cases it is sensitive, isn't it?

DR. DELLARCO: In most cases, it is. But again, all compartments are looked at and selected.

DR. POPE: I didn't say brain cholinesterase inhibition. I said cholinesterase

inhibition.

DR. DELLARCO: Pardon?

DR. POPE: Cholinesterase inhibition in any tissue. Are there single compounds that are regulated on the basis of a noncholinesterase most critical endpoint?

DR. DELLARCO: In the case of chlorpyrifos, the FQPA safety factor was retained. Although the R F D endpoints were based on cholinesterase inhibition, a 10X factor was retained because of other toxicities that were observed in the developing nervous system that may not have been due to the cholinergic system.

DR. POPE: But the RFDs were all based on cholinesterase inhibition?

DR. DELLARCO: I think mostly all the RFDs. Karl, can you --

DR. BAETCKE: This is Karl Baetcke. There may be a few exceptions. But for most, it is based on cholinesterase. What I can't recall is when you get into the chronic studies, there may be other endpoints for the long term.

DR. DELLARCO: But also, you have to keep in mind when the FQPA decisions were made for certain OPs, a factor, whether it was 10 or maybe 3X, was retained because of the consideration of other toxicities.

DR. ROBERTS: Dr. Reed.

DR. REED: While we were looking at the single and the modical chemical exposure, I was curious to know if by applying different uncertainty factor to single versus to modical, would it create something so that -- I think people conceptually are looking for cumulative risk being greater than single chemical risk, because conceptually it is cumulative, meaning you have other exposures that come into play, but are there situations where you might have risk for single chemical turn out to be greater than cumulative risk.

And is that sort of confusing in terms of that comparison.

MS. MULKEY: It depends on whether you are looking at your cumulative risk before or

after you've regulated your single chemical. That's part of what makes that question complicated.

I suppose it is -- our effort is to have completed at least enough work on the single chemical that we understand its entire profile.

In most instances, we have not only completed the risk assessment for the single chemical, we have completed risk management.

This is more of a science question, I probably shouldn't try to answer it. I think it is theoretically possible that you could have an endpoint in a single chemical that was far more sensitive than your common mechanism endpoint. So you could have a single chemical where your risk gave you much greater concern than the cumulated -- the risk from the cumulated exposure of the class as it related to the common mechanism endpoint.

I don't know whether that theoretical prospect exists for this class of chemicals.

DR. ROBERTS: I think that's right.

DR. MATSUMURA: Theoretically, yes, many OPs can affect the carboxylesterases. There are some report clearly to show those joint kind of actions. Iso malathion, for instance, is going to affect on the purity of a chemical. One component of the same compounds or different OPs (ph) can inhibit the carboxylesterase.

I'm quite sure Dr. Padilla has addressed that, too, right? Some compounds could affect the A esterases, too, via competition. So interactions are there, theoretically.

DR. ROBERTS: This is the last call for comments.

Seeing none, I would like to thank the members of the panel for their time and effort in preparing for this meeting, for their excellent comments and discussions.

I would like to thank the agency for, obviously, their very hard work in preparing this analysis, their presentations and very useful and candid discussions with us on the technical issues.

And of course I would like to thank the SAP support staff for putting this meeting together. There are a lot of logistical details associated with assembling a panel, getting the materials to the panel, getting everybody here and so forth. They do a terrific job for us. I would like to thank all of them for that.

We're going to close this session now. And I would ask the members of the panel to meet just to cover some administrative details in terms of preparing the minutes from this meeting.

Is there any other announcements or anything anyone would like to say before we finish for the day?

MR. LEWIS: Just briefly, I want to thank Dr. Roberts for serving as chair for our meeting over the past few days, and again, thanking the panel for your thoughtful deliberations over the past two days.

The panel will now work in preparing its minutes for the discussion for the past two days. We anticipate having the report, the minutes

available in approximately two to three weeks.

Thank you.

DR. ROBERTS: If there are no further announcements, this session of the FIFRA Scientific Advisory Panel is now closed.

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[Whereupon, at 2:30 p.m., the meeting concluded.]

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I, Frances M. Freeman, Stenotype Reporter, do hereby certify that the foregoing proceedings were reported by me in stenotypy, transcribed under my direction and are a verbatim record of the proceedings had.

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